

FIG. 1B

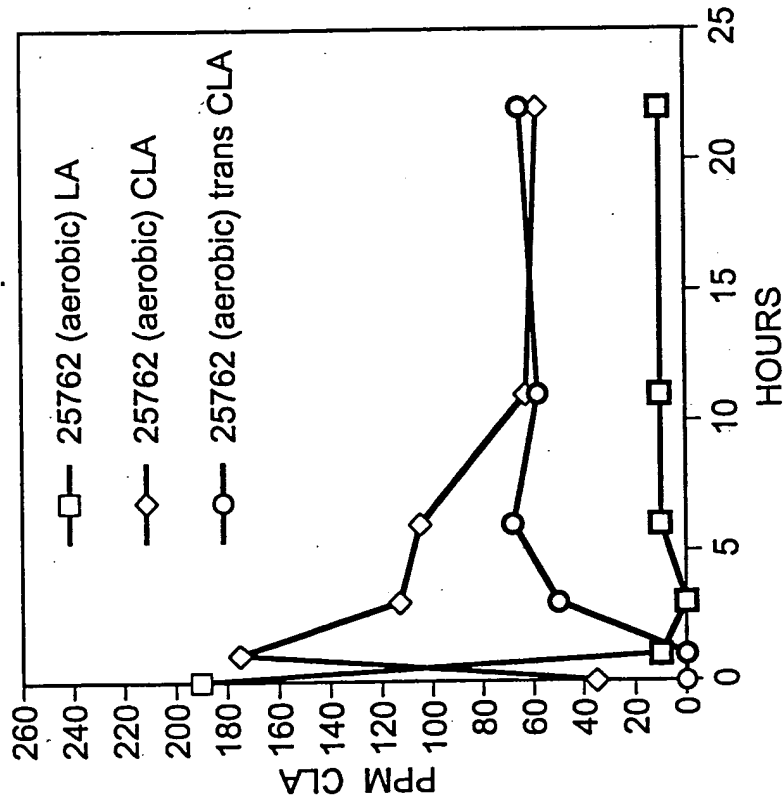


FIG. 1A

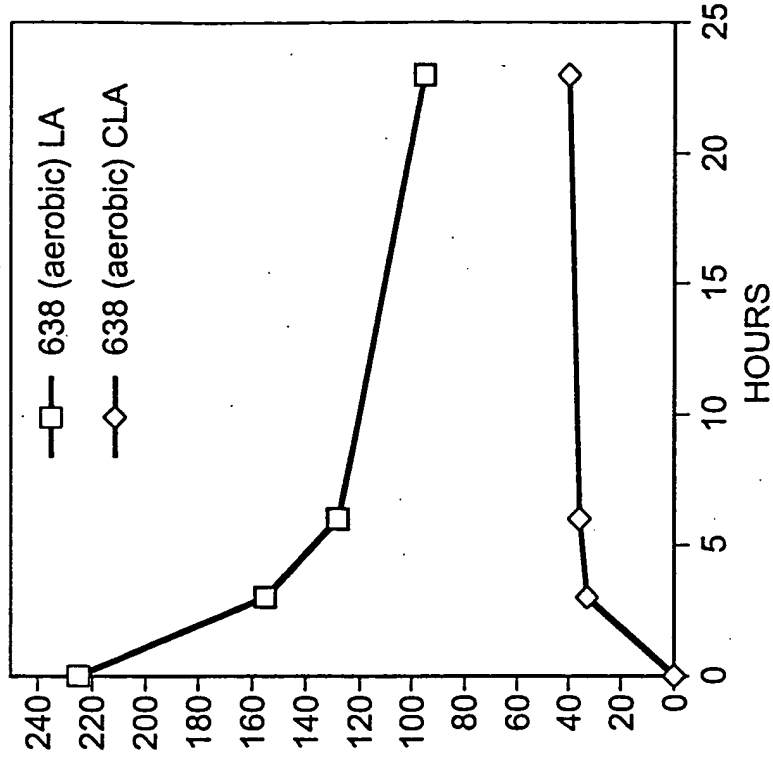


FIG. 2B

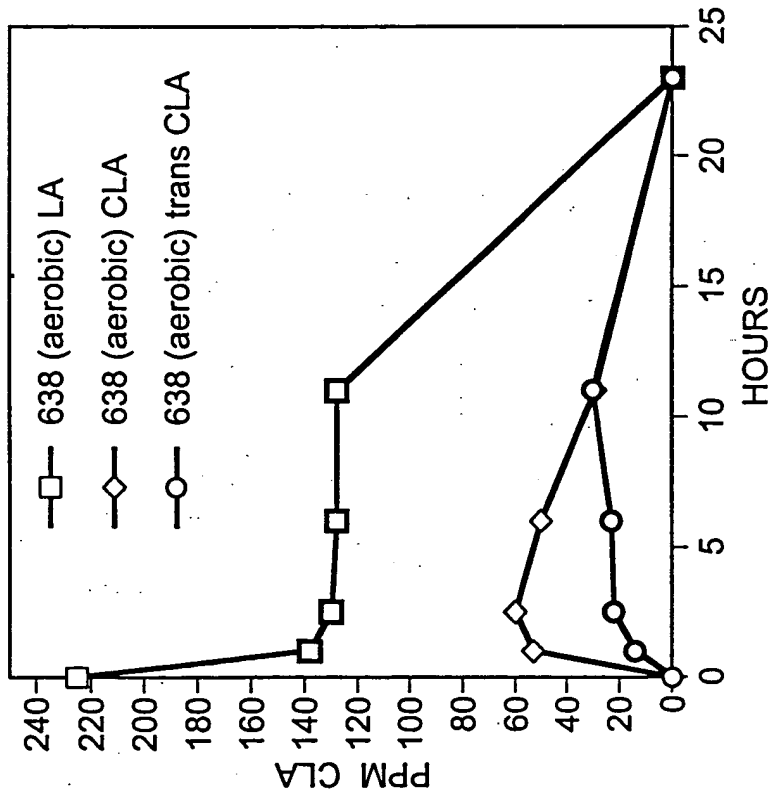


FIG. 2A

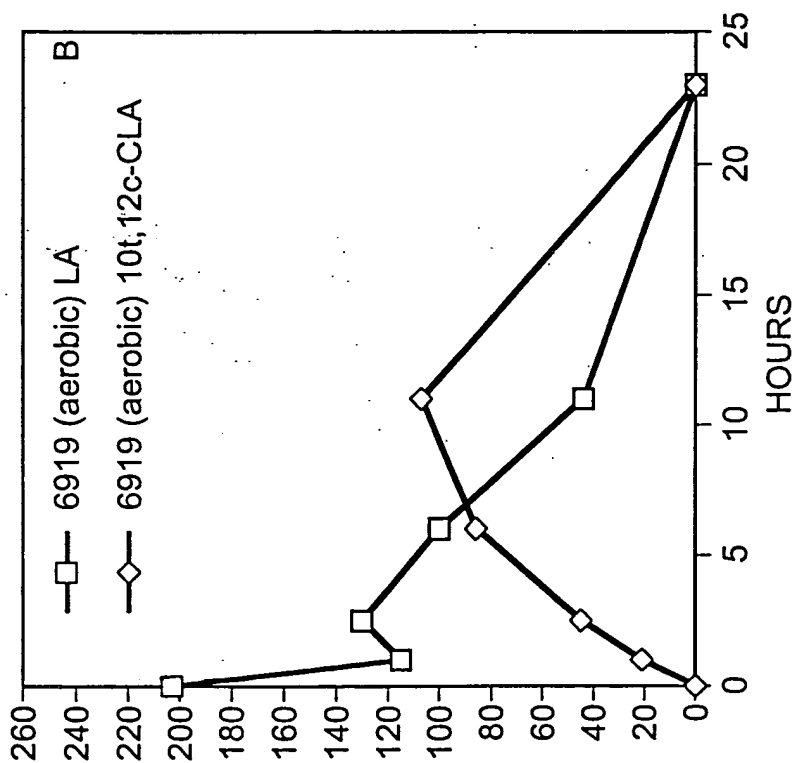


FIG. 3B

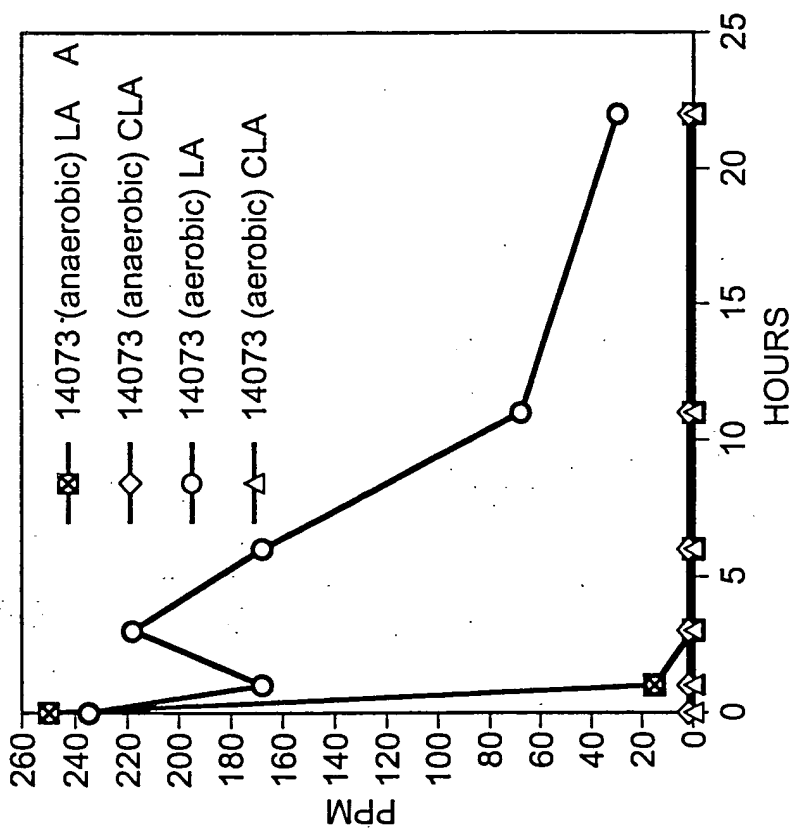


FIG. 3A

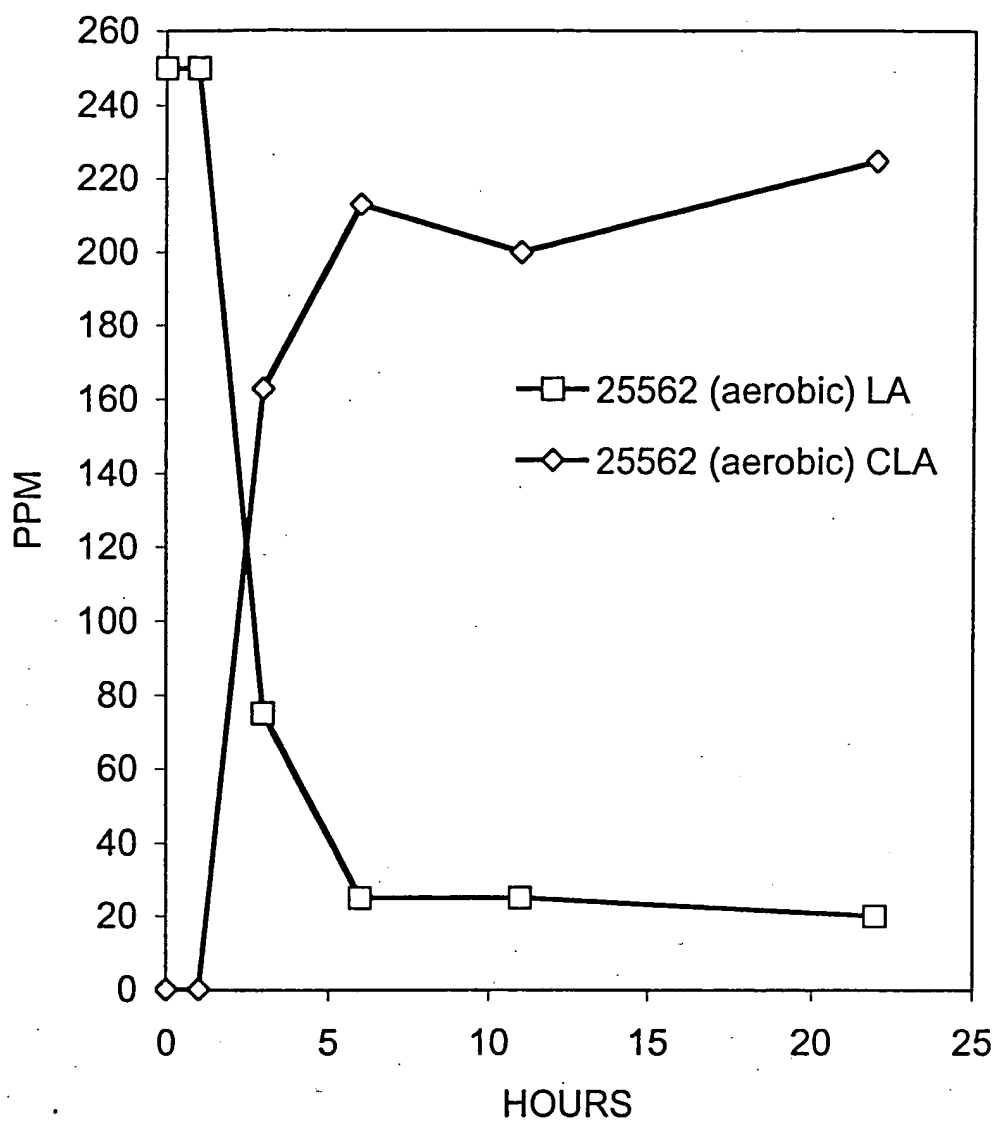


FIG. 4

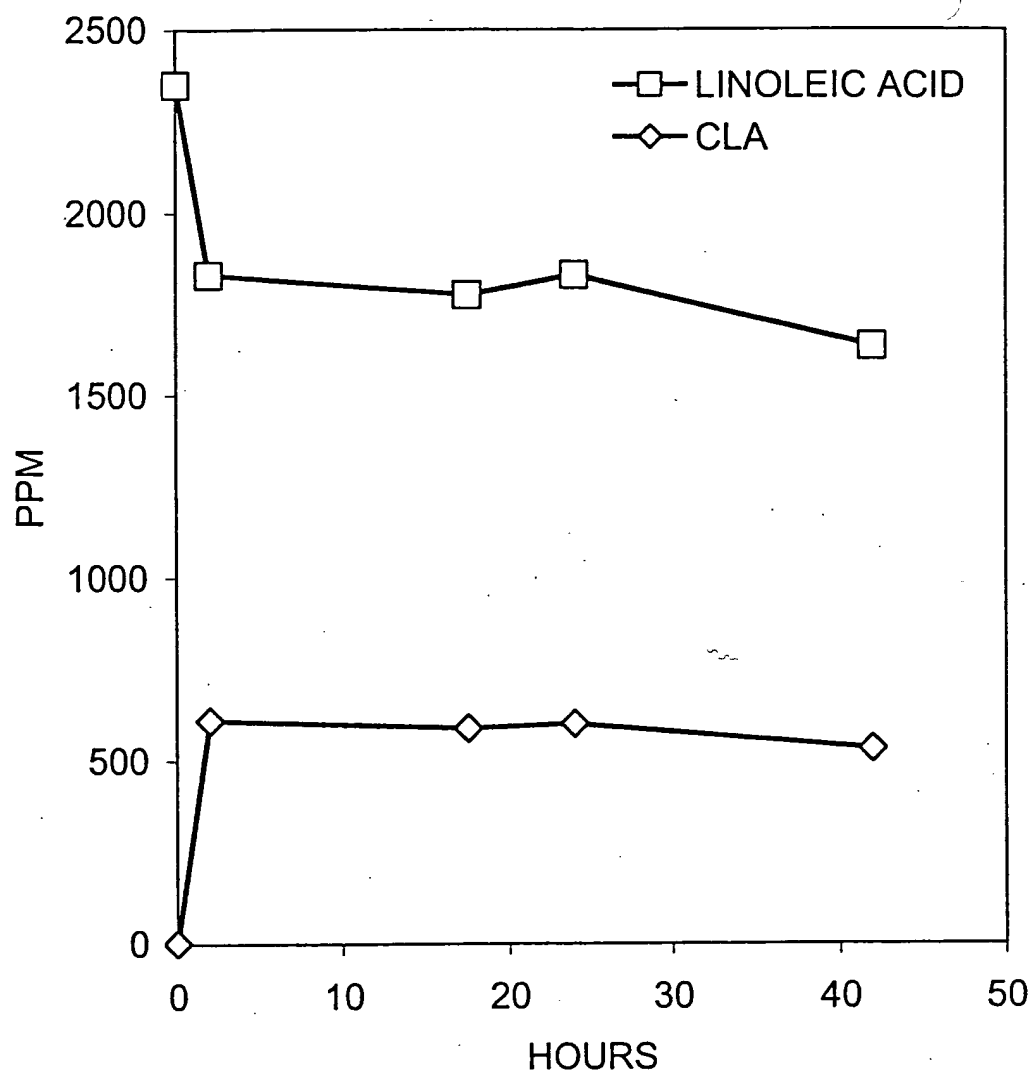


FIG. 5

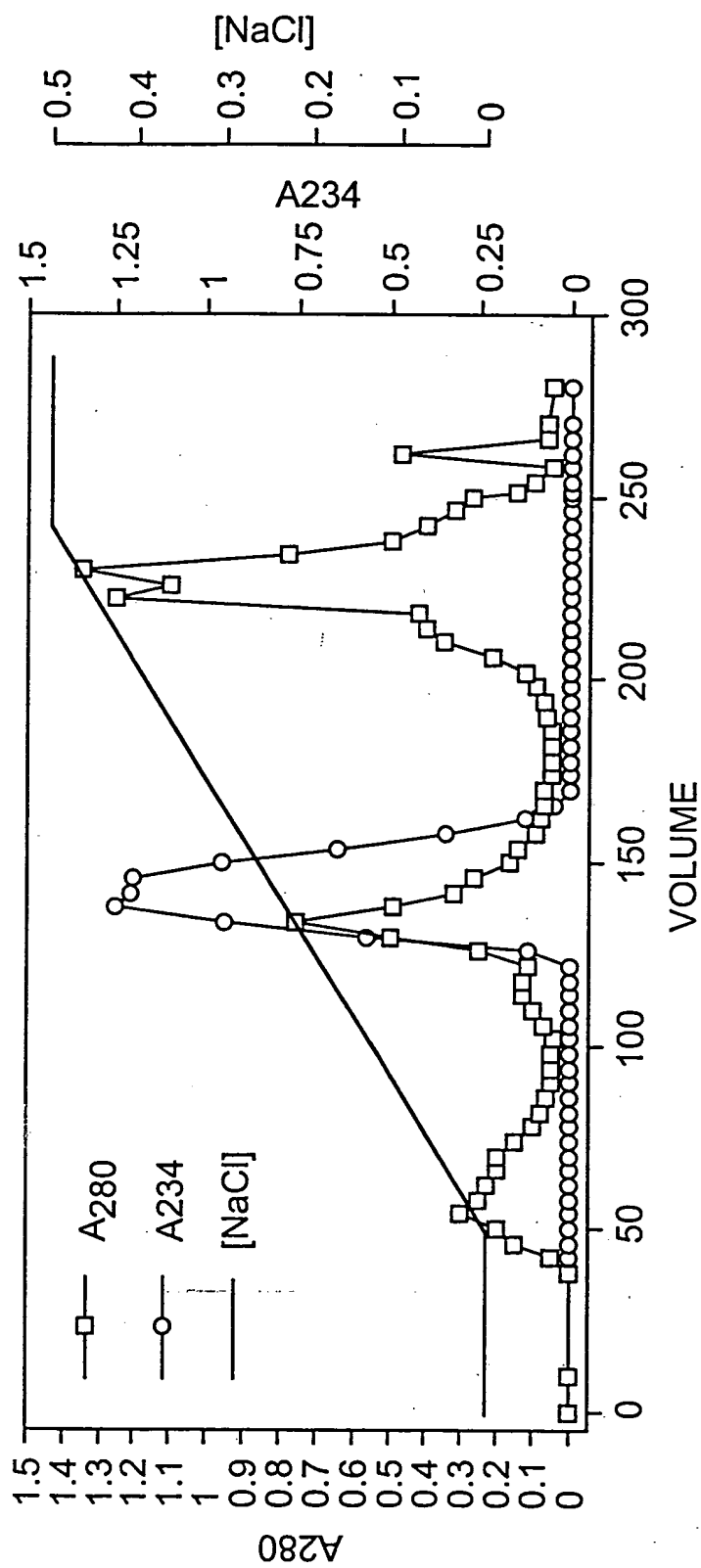


FIG. 6

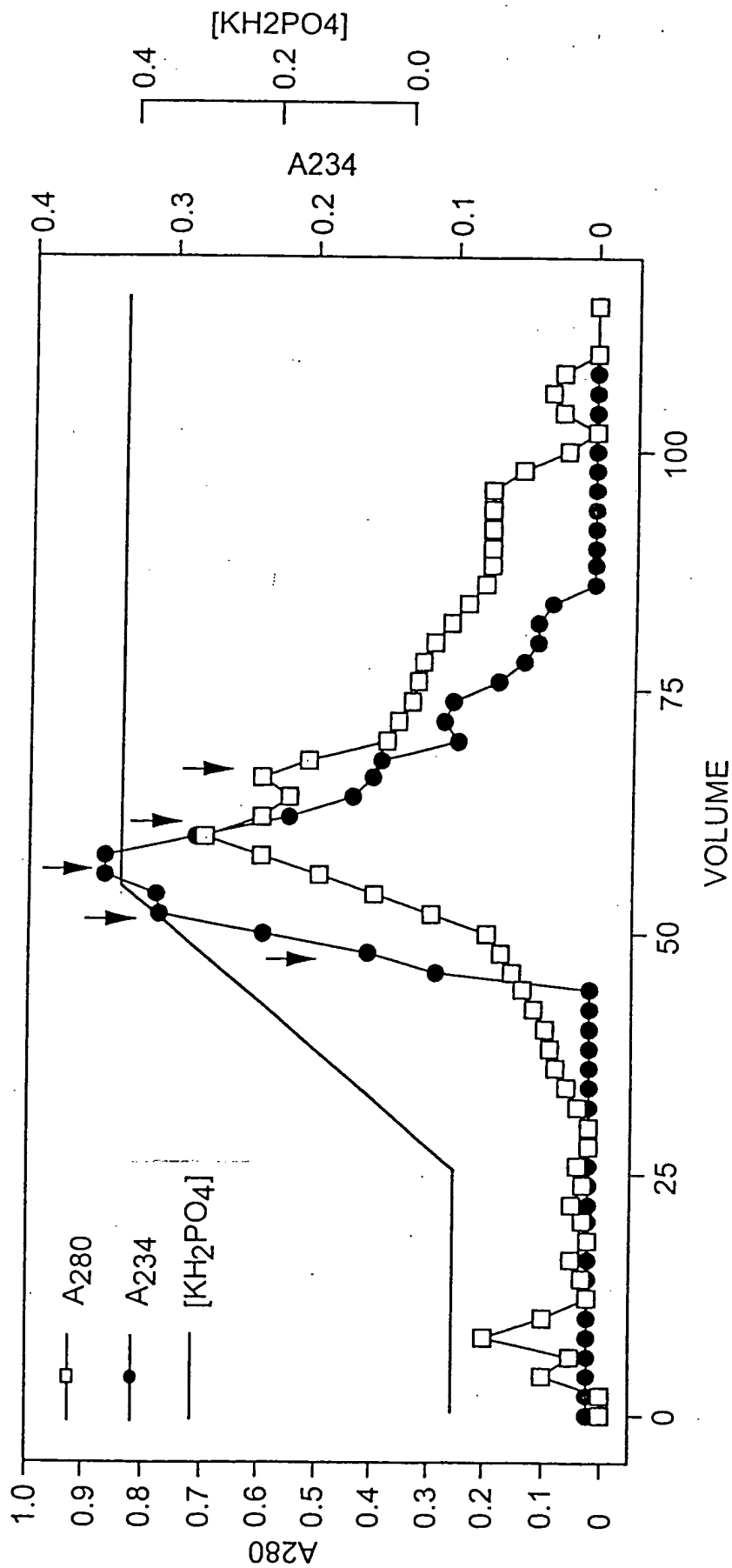


FIG. 7

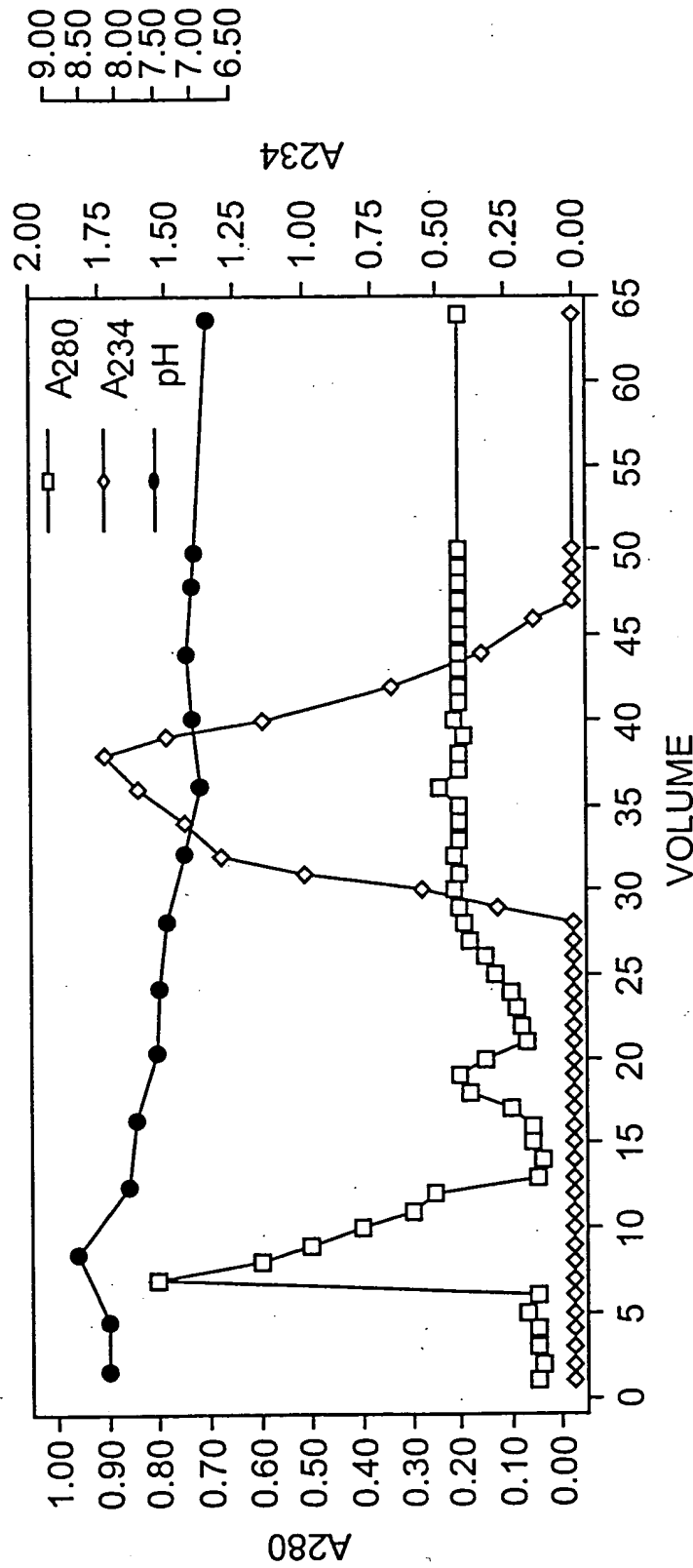


FIG. 8



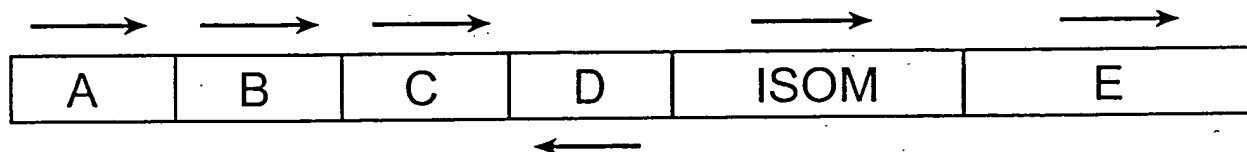


FIG. 9

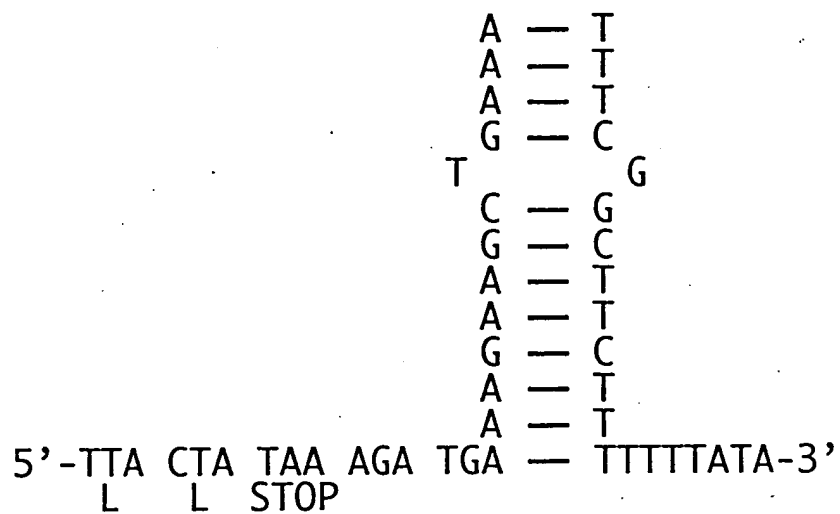
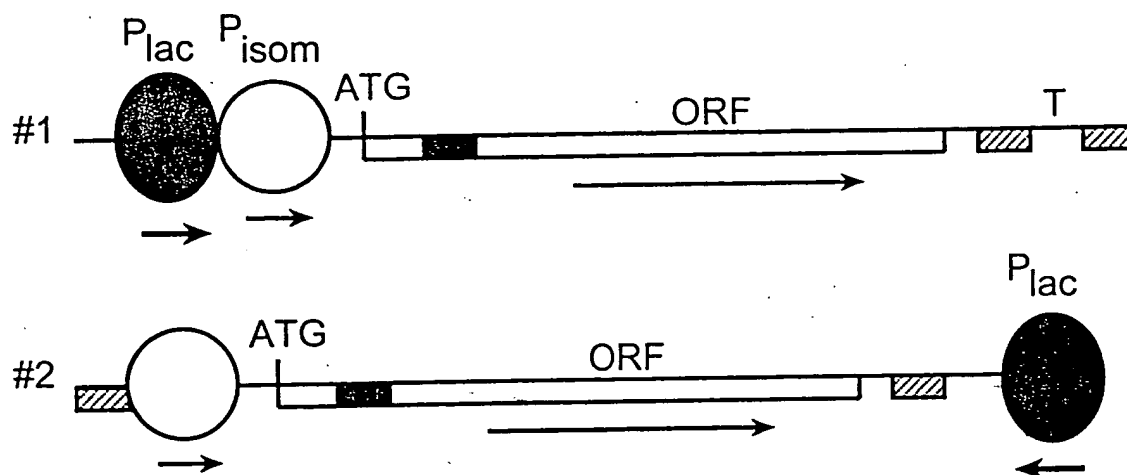
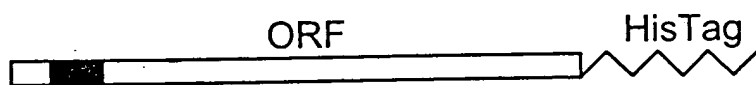


FIG. 10

## Tested Two Constructs



## New Construct #3:



## New Construct #4:

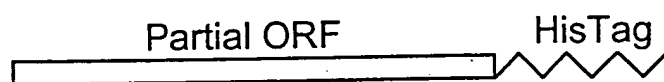


FIG. 11

Expression System:

HapII promoter

LAT promoter

— with the secretion signal peptide

— without the secretion signal peptide

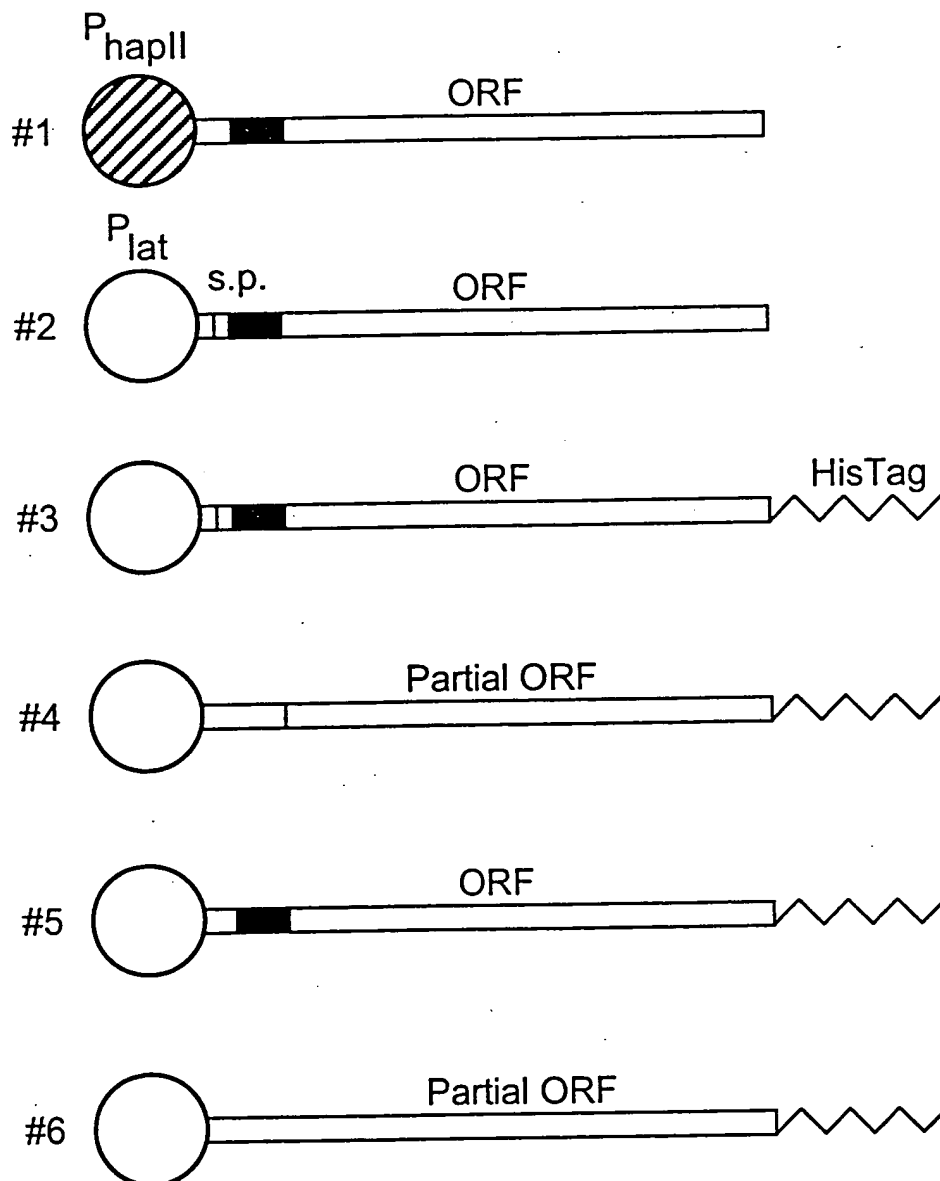


FIG.12

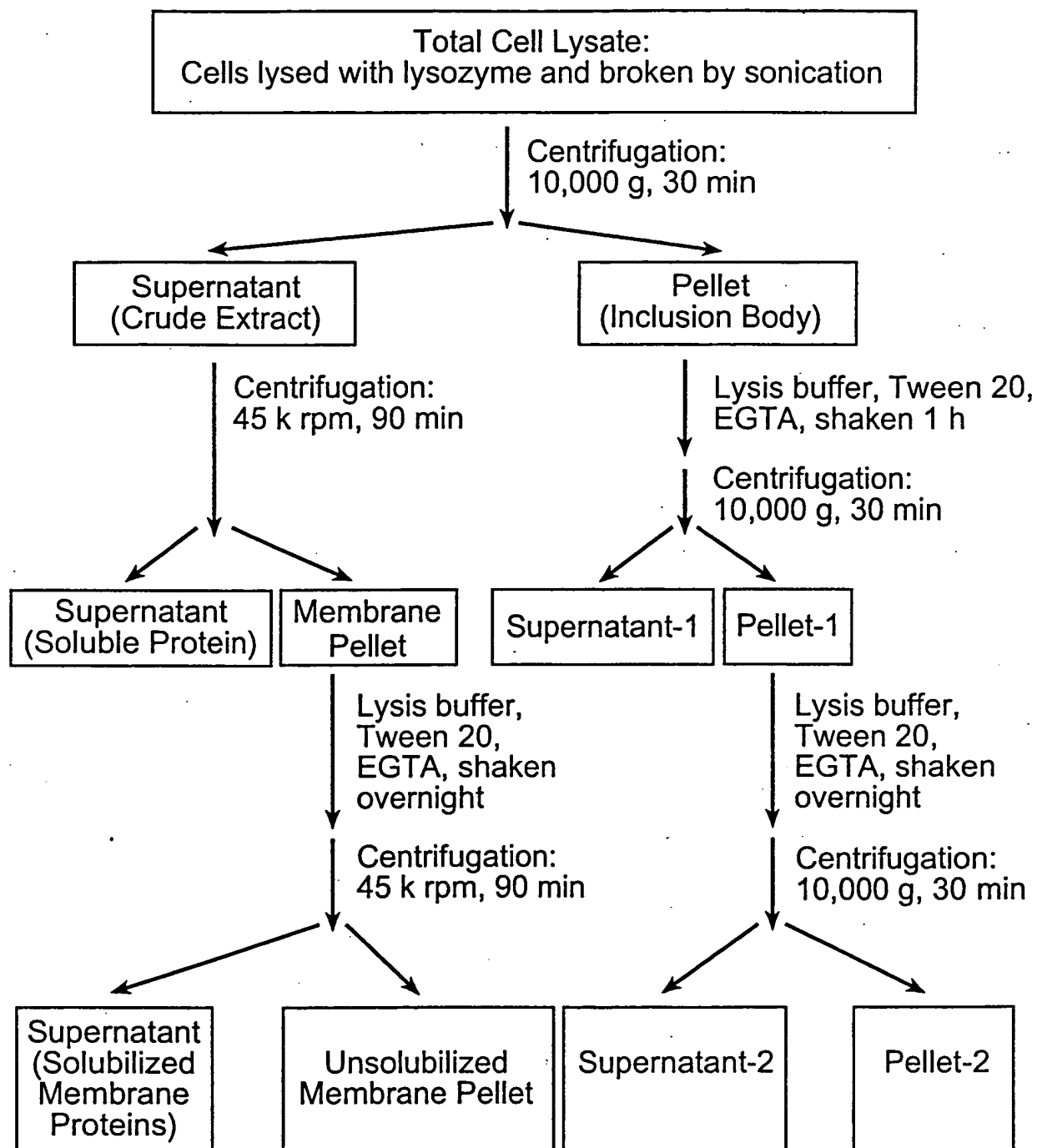


FIG. 13

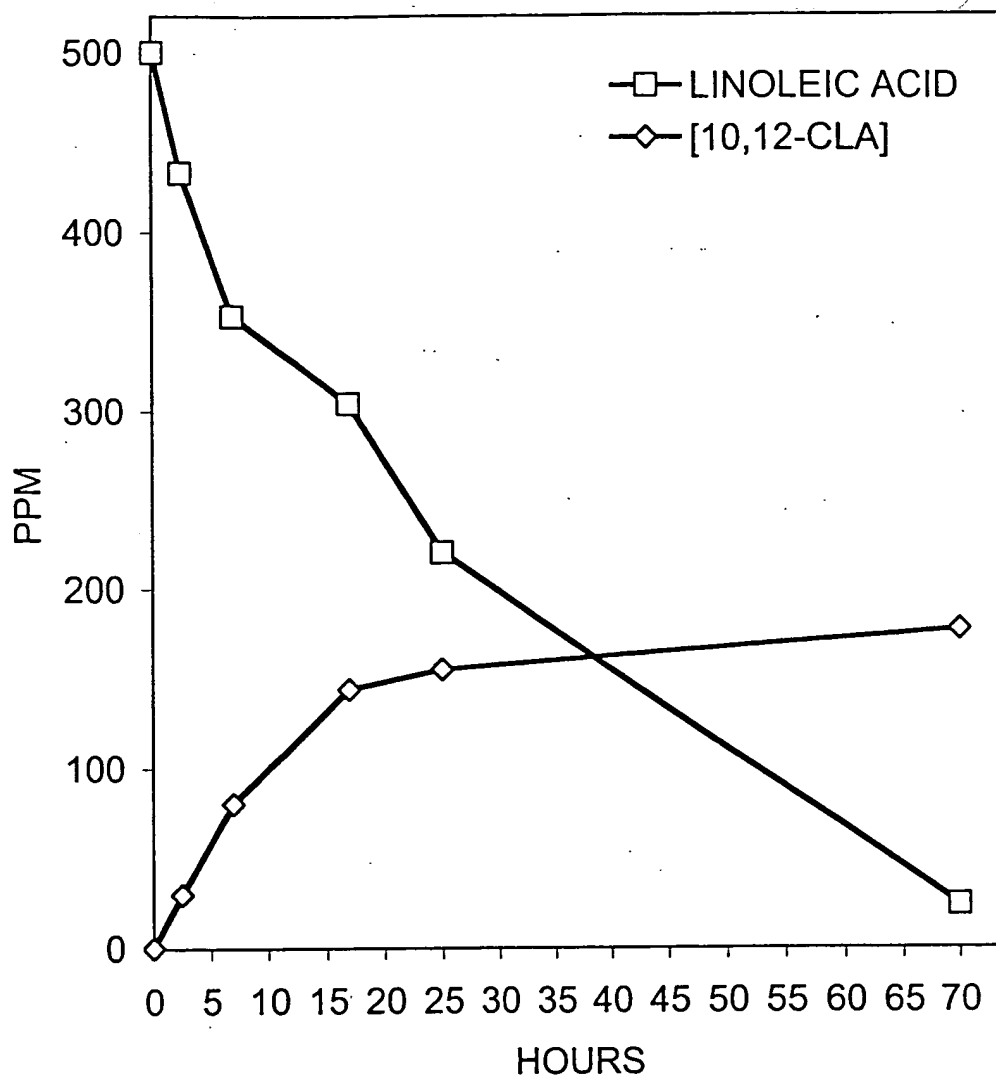


FIG. 14

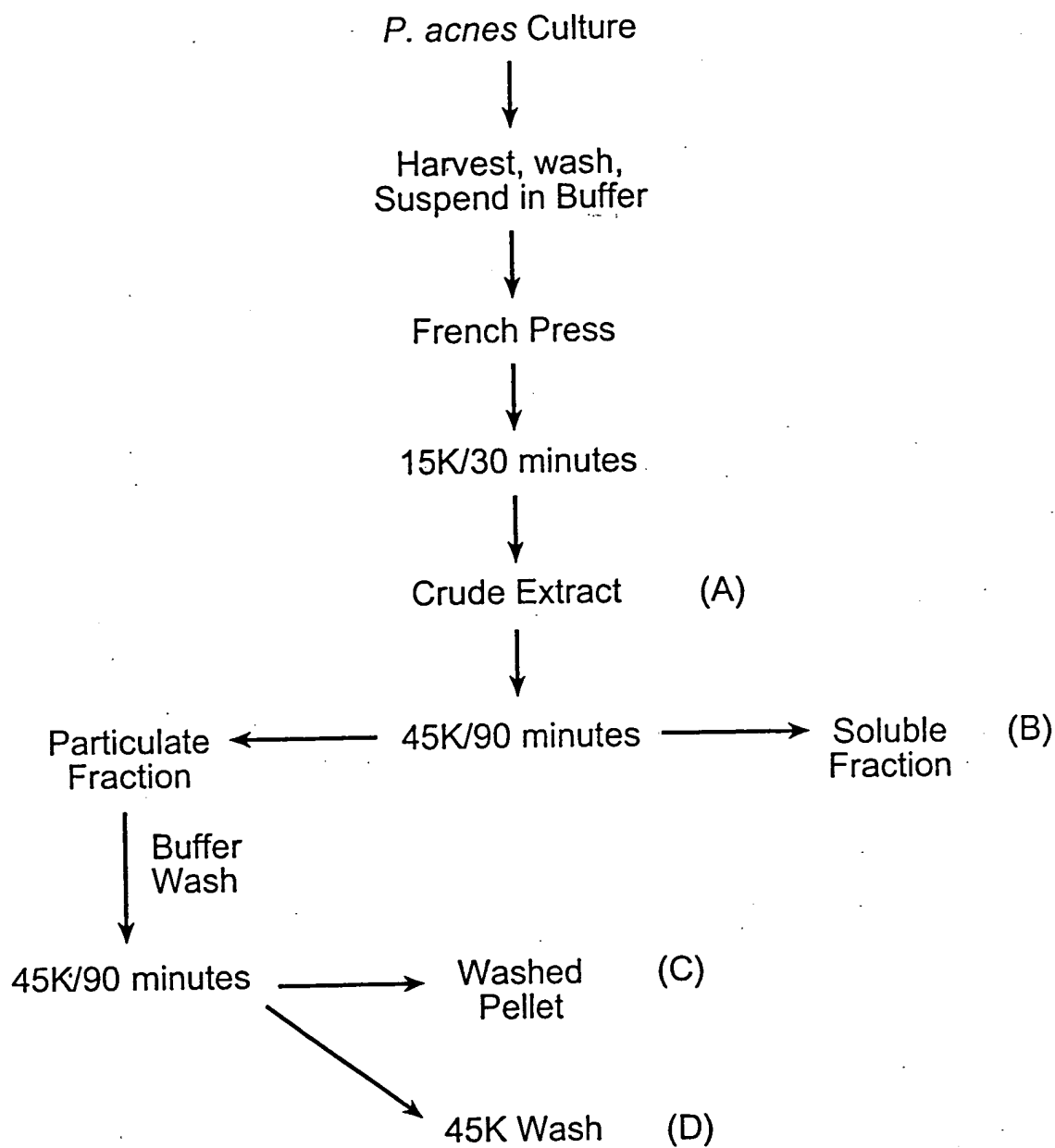


FIG.15

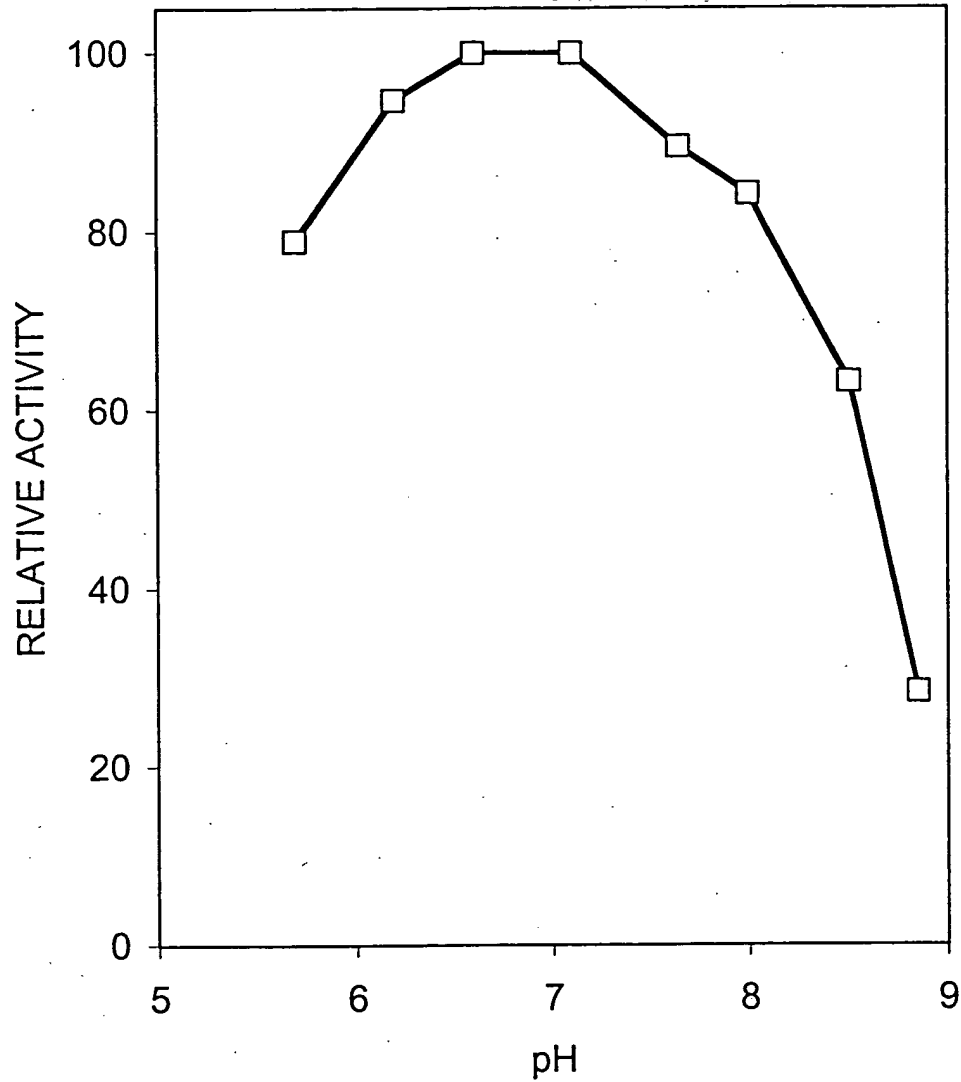


FIG. 16



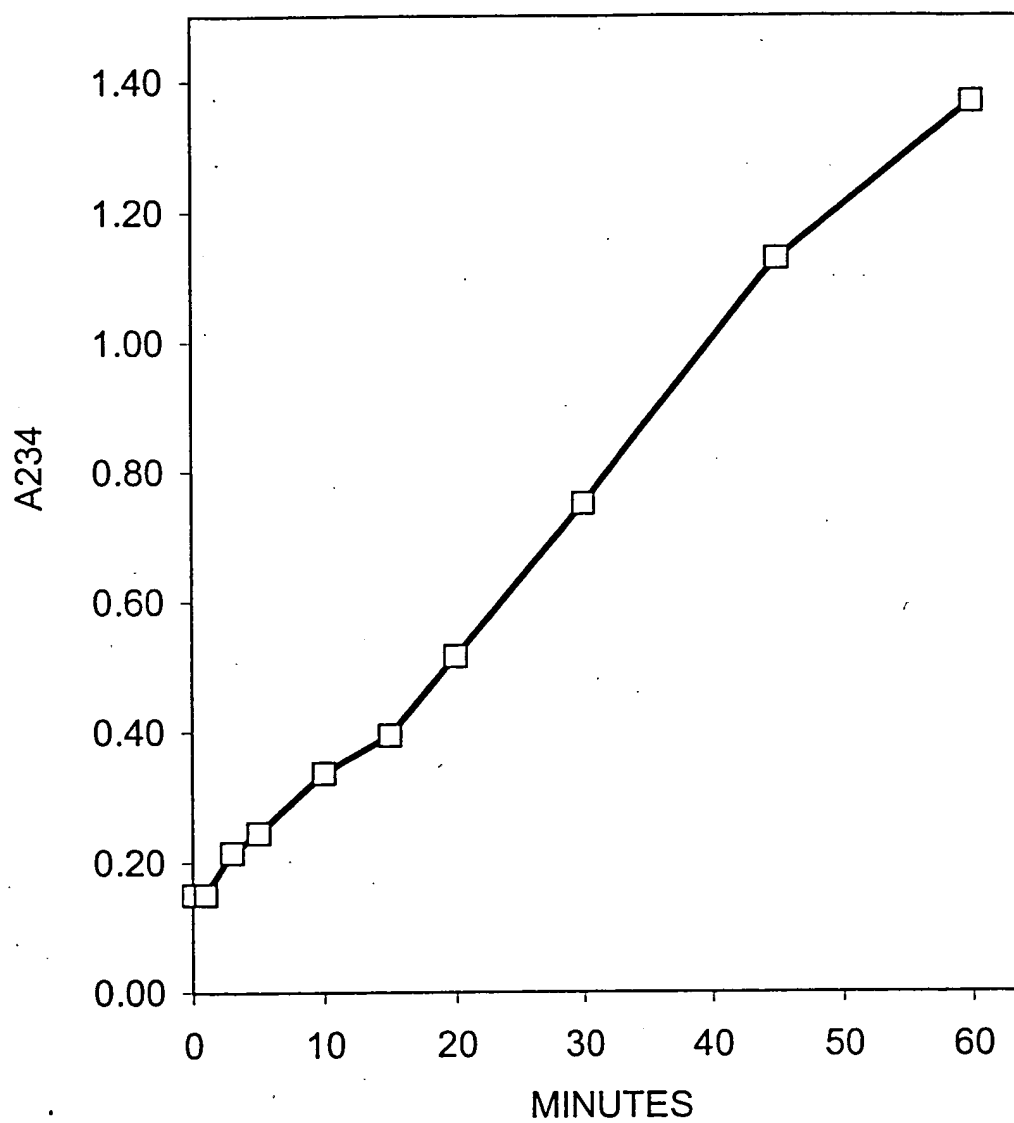


FIG. 17

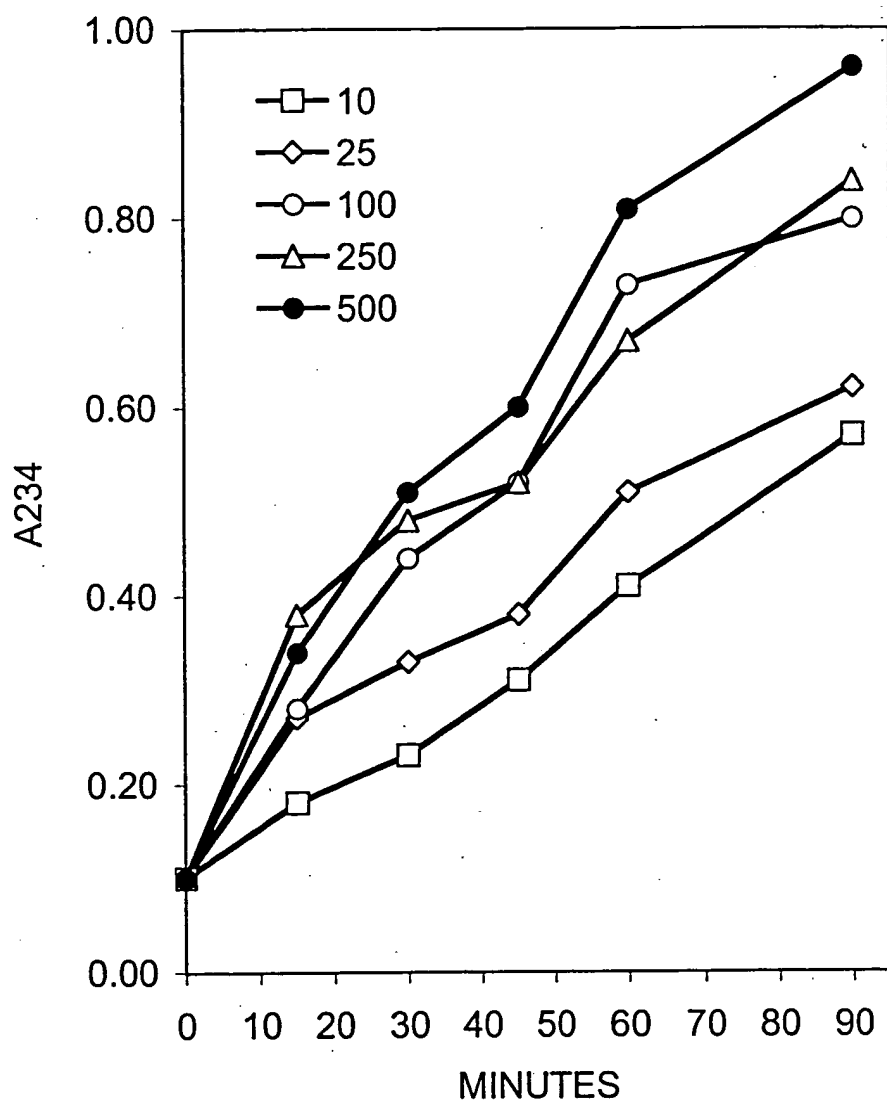


FIG. 18

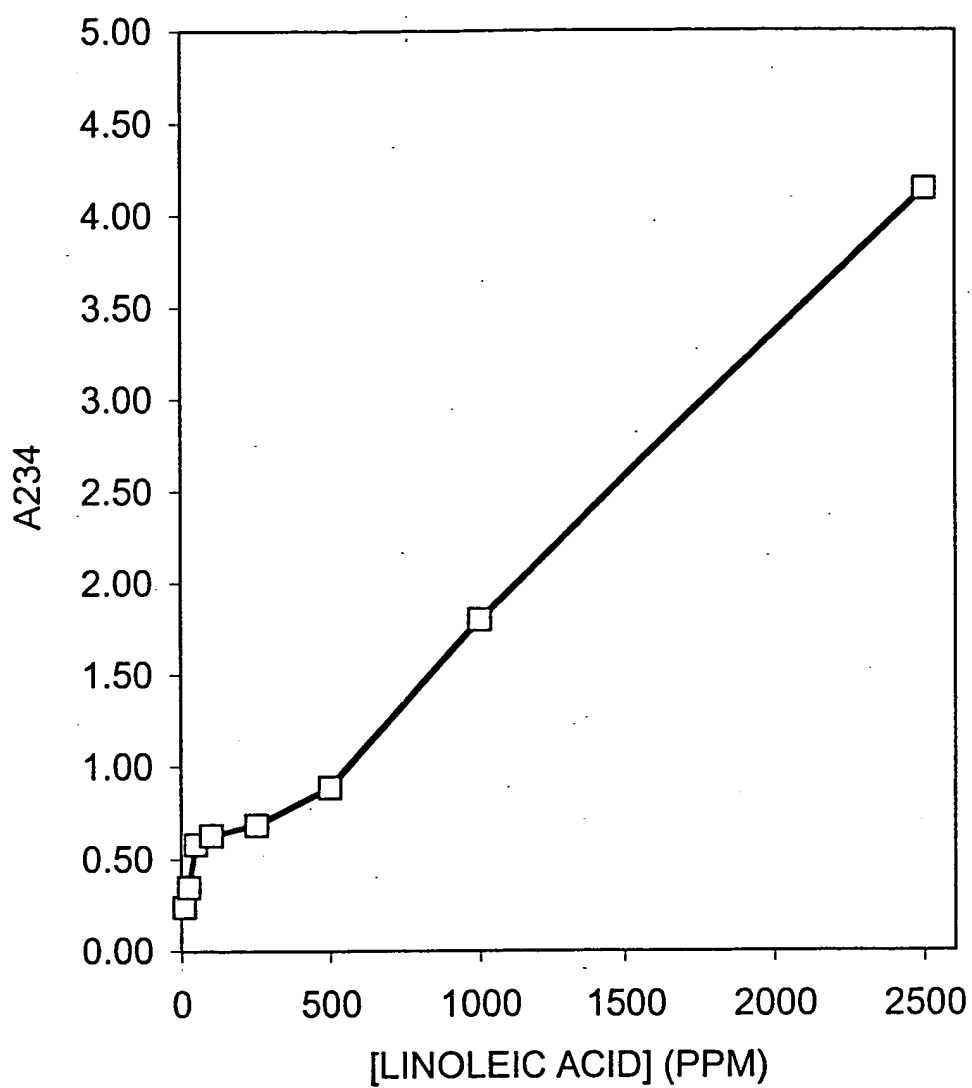


FIG. 19

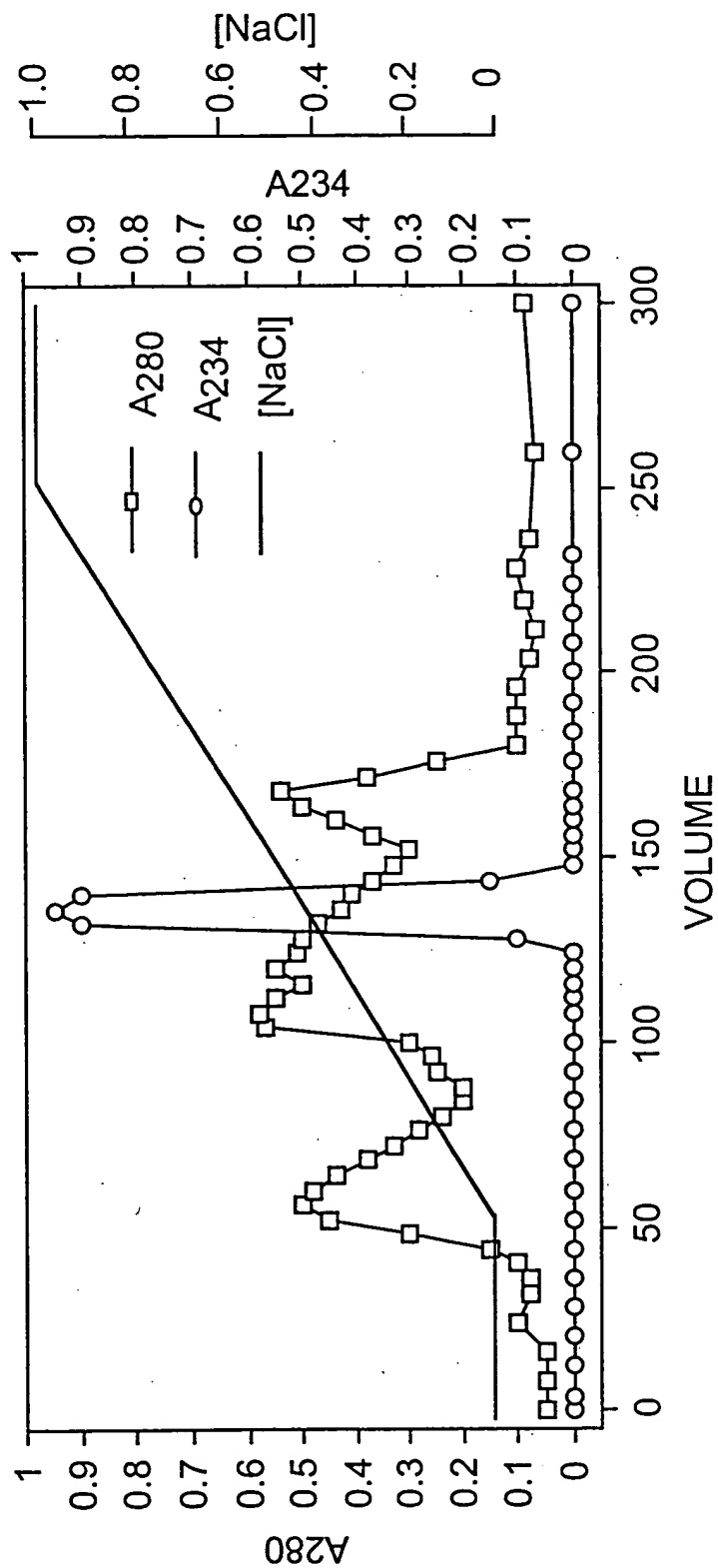


FIG. 20

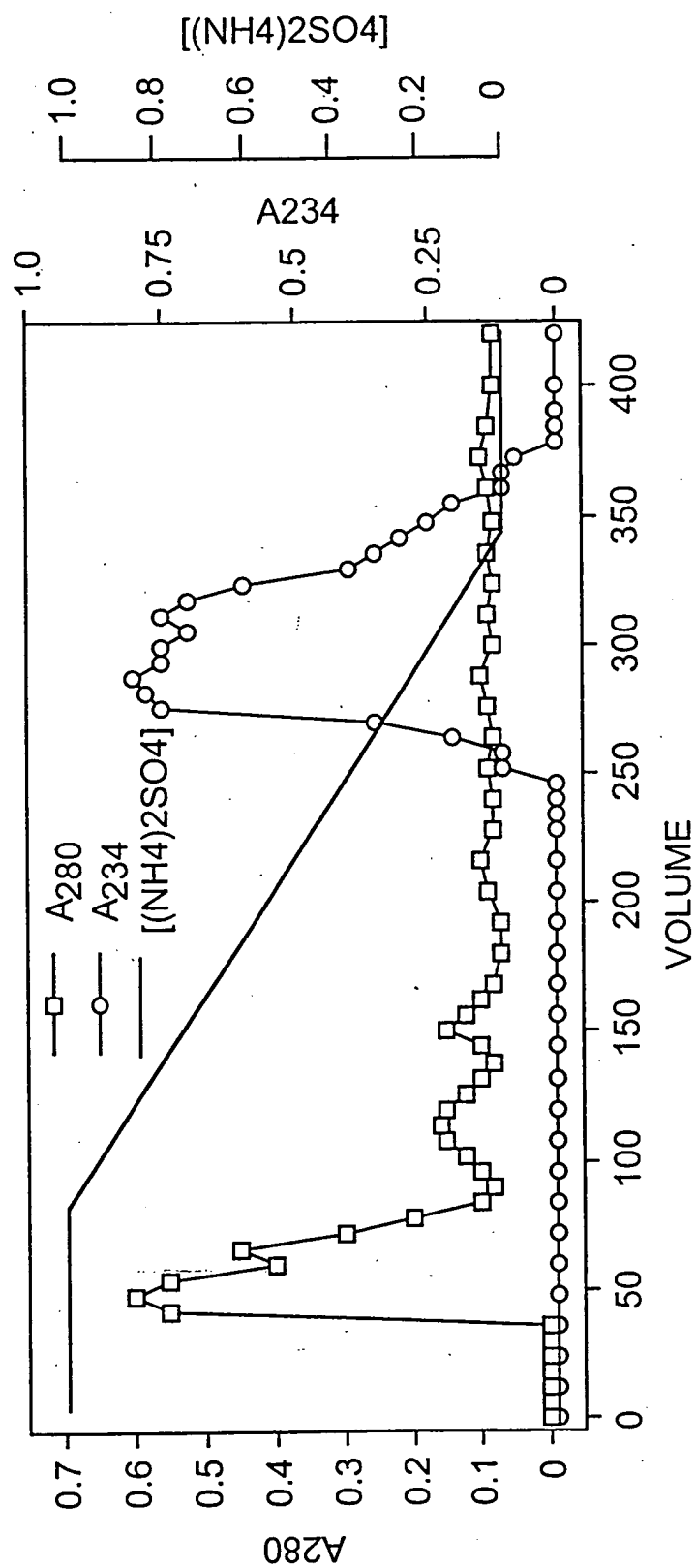


FIG. 21

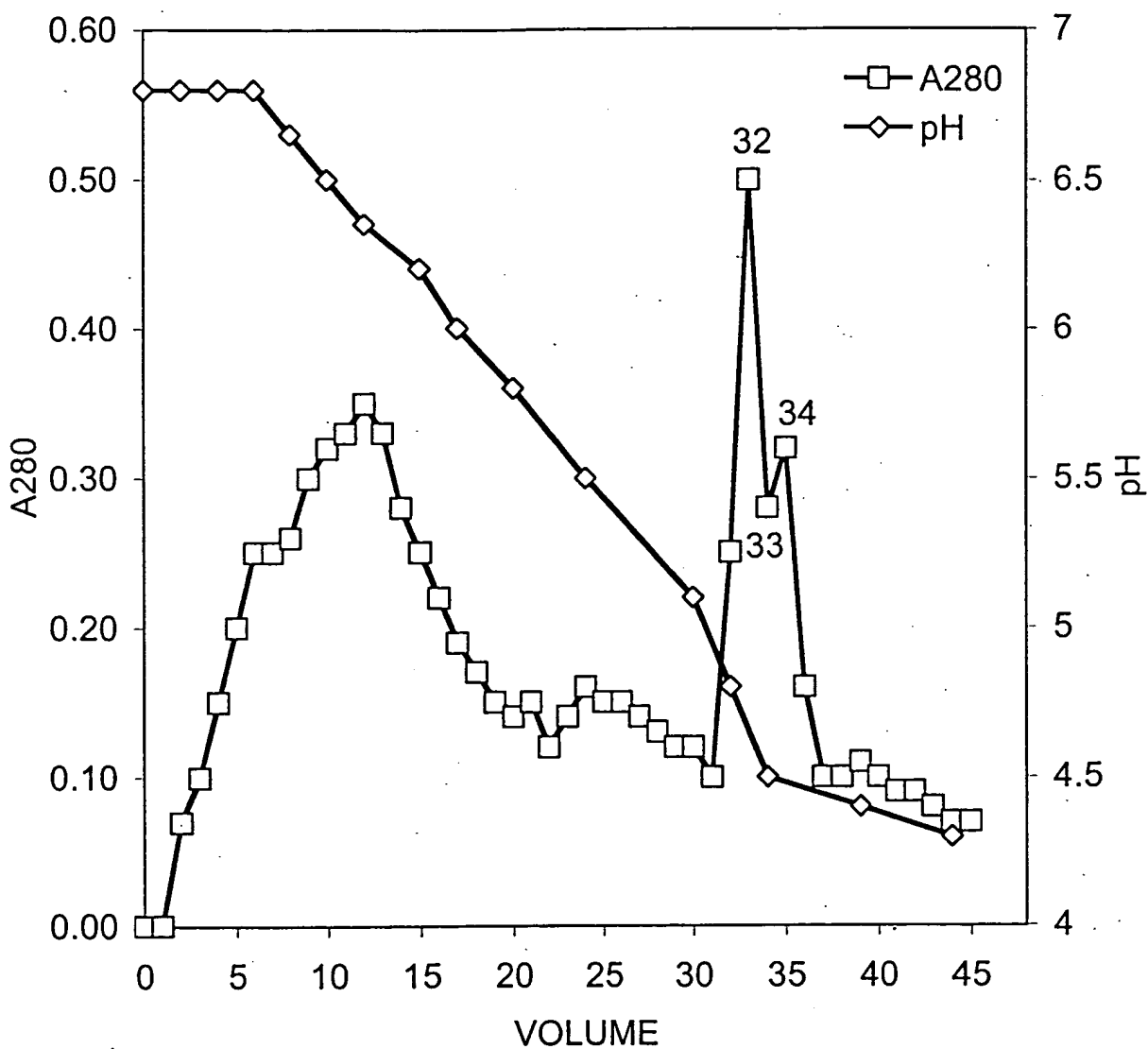


FIG. 22

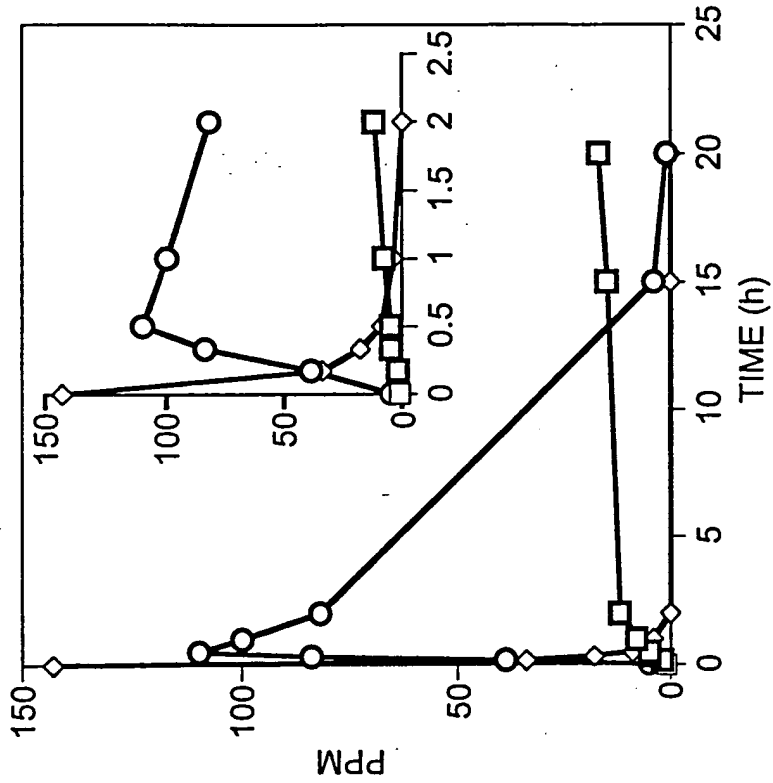


FIG. 23B

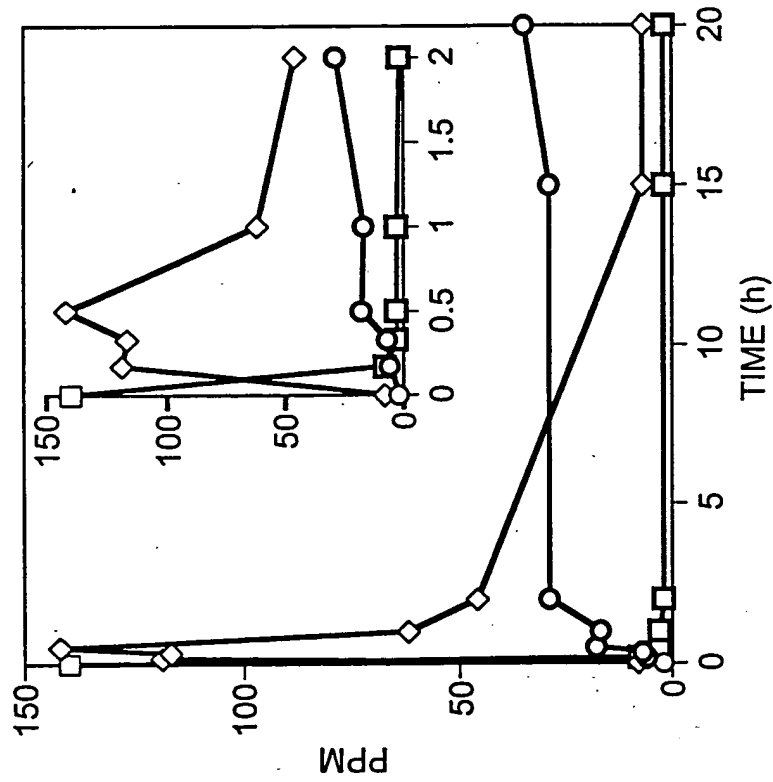


FIG. 23A

Data whole/37°C /Aerobic

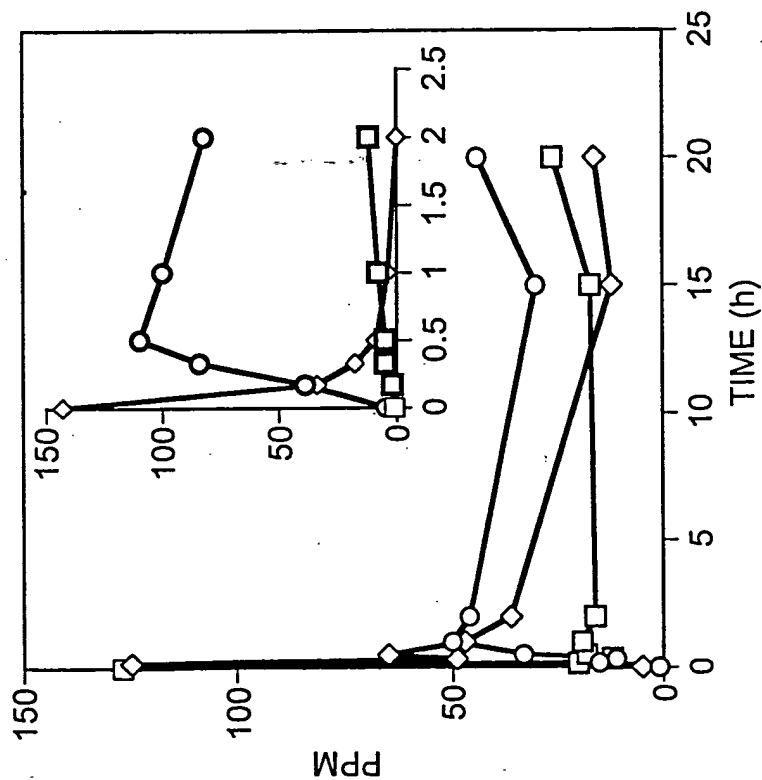


FIG. 23C

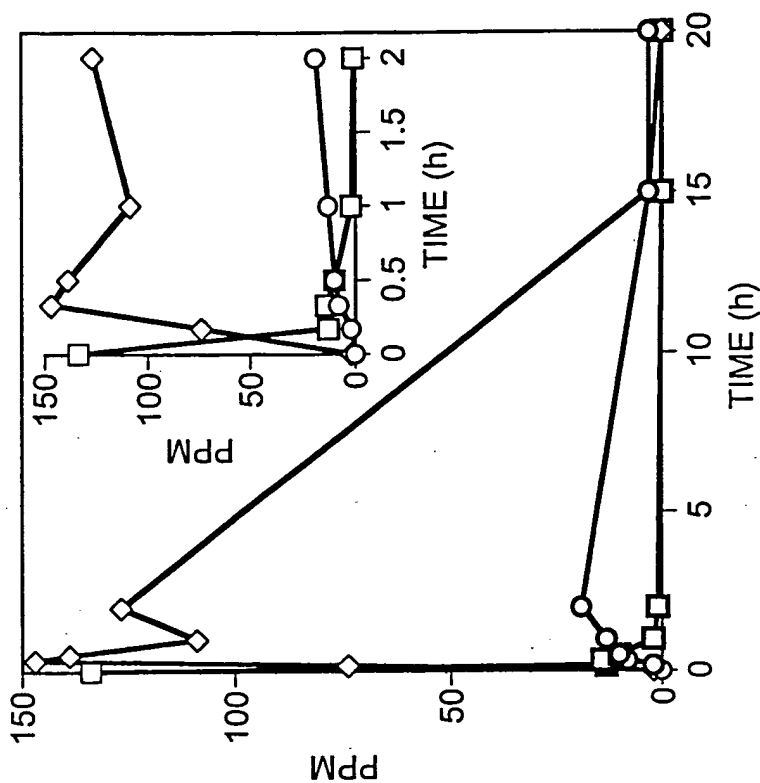


FIG. 23D



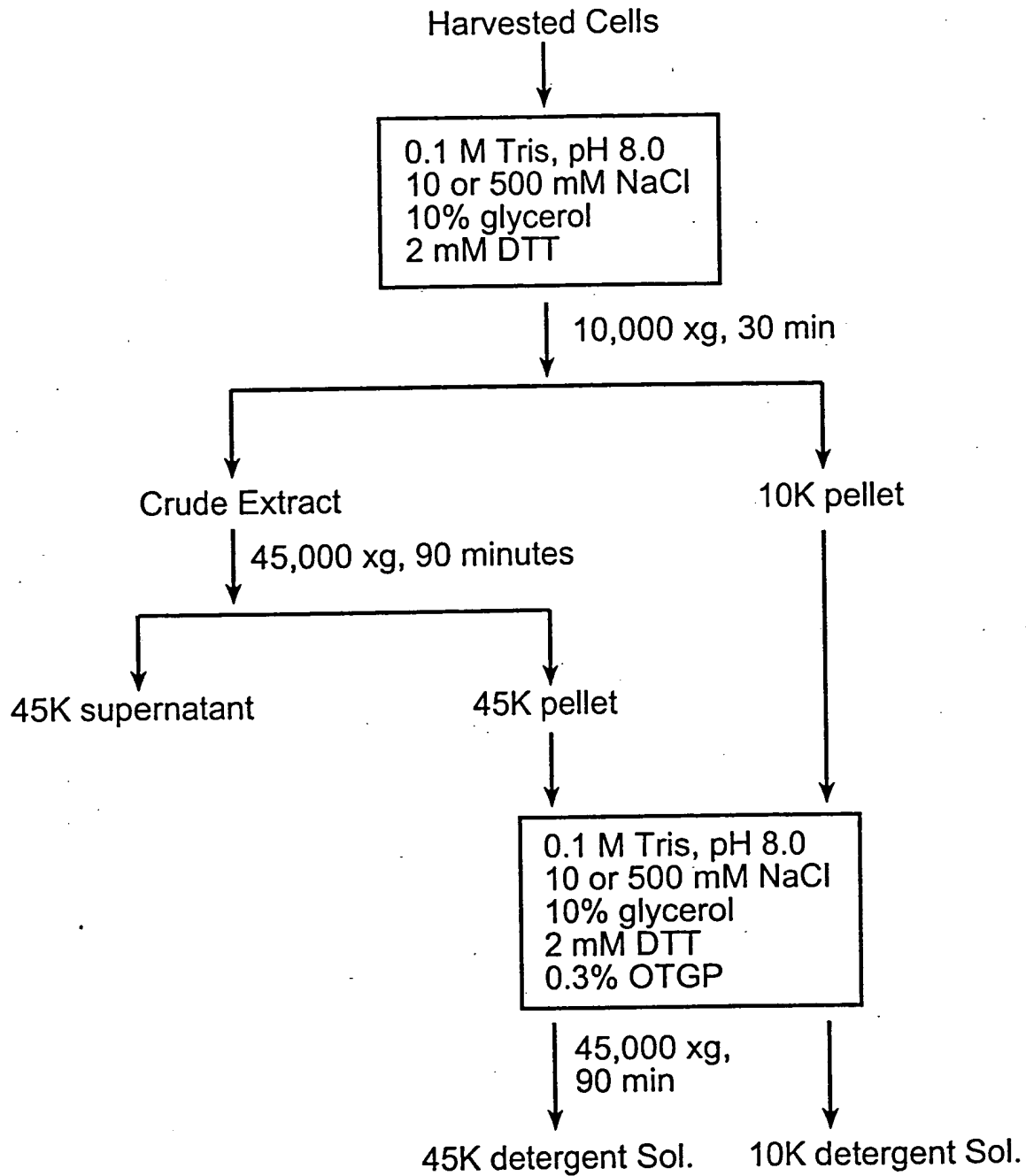


FIG. 24

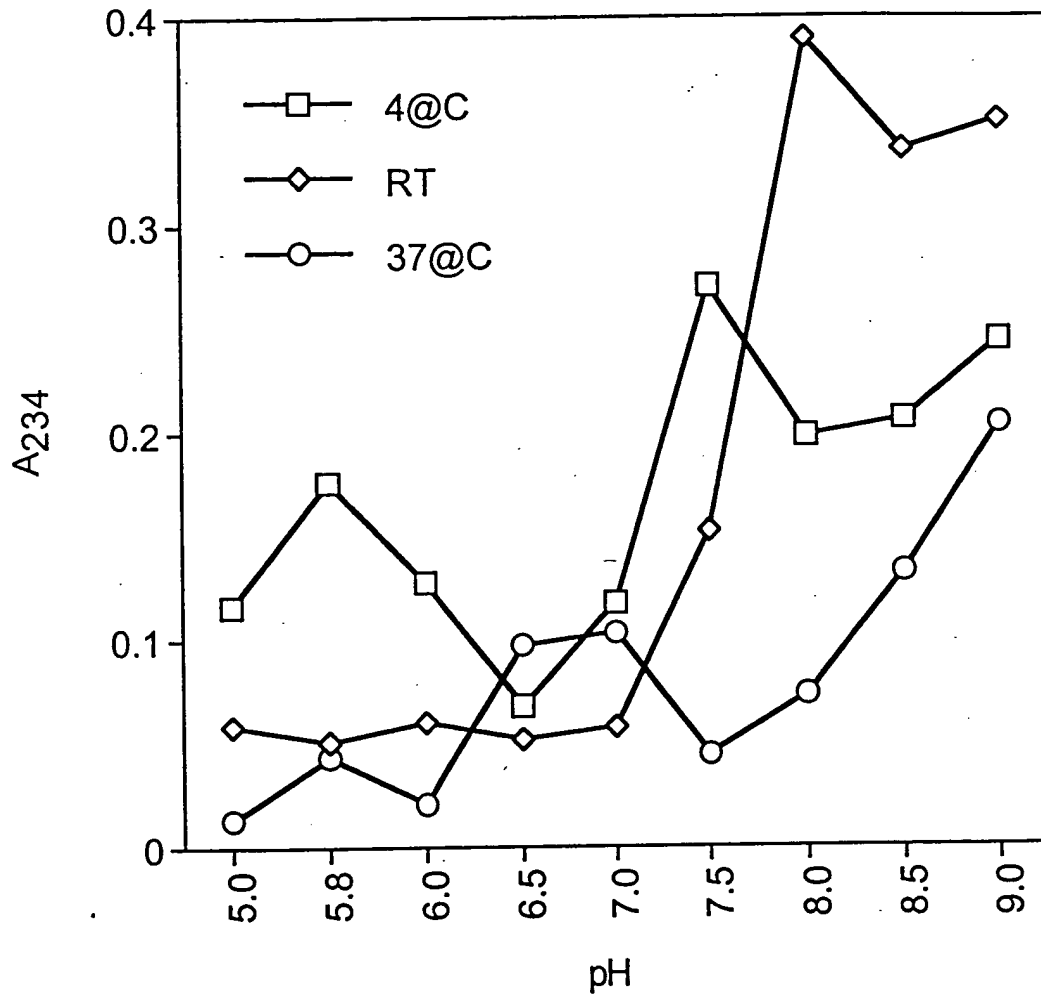


FIG. 25

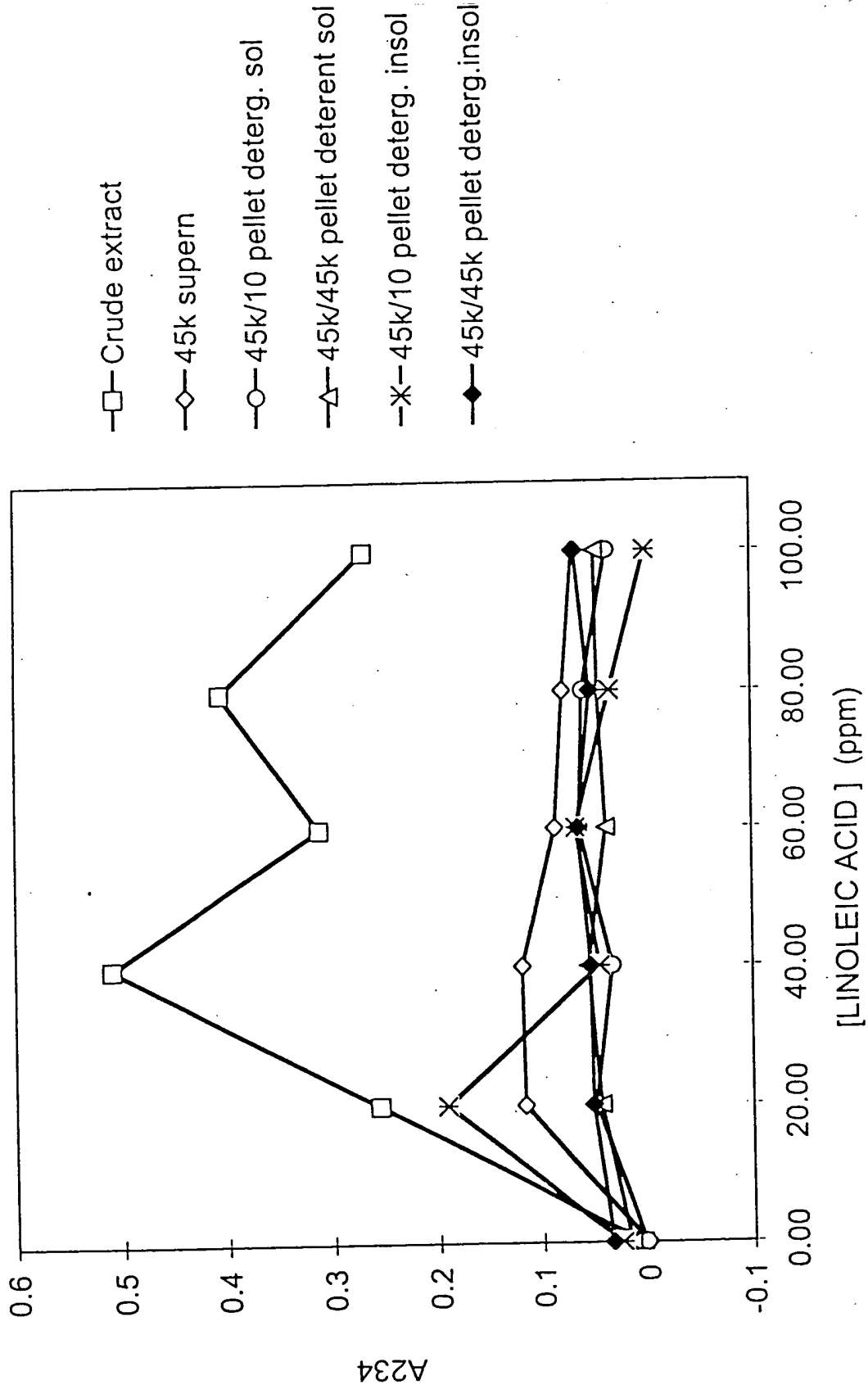


FIG. 26

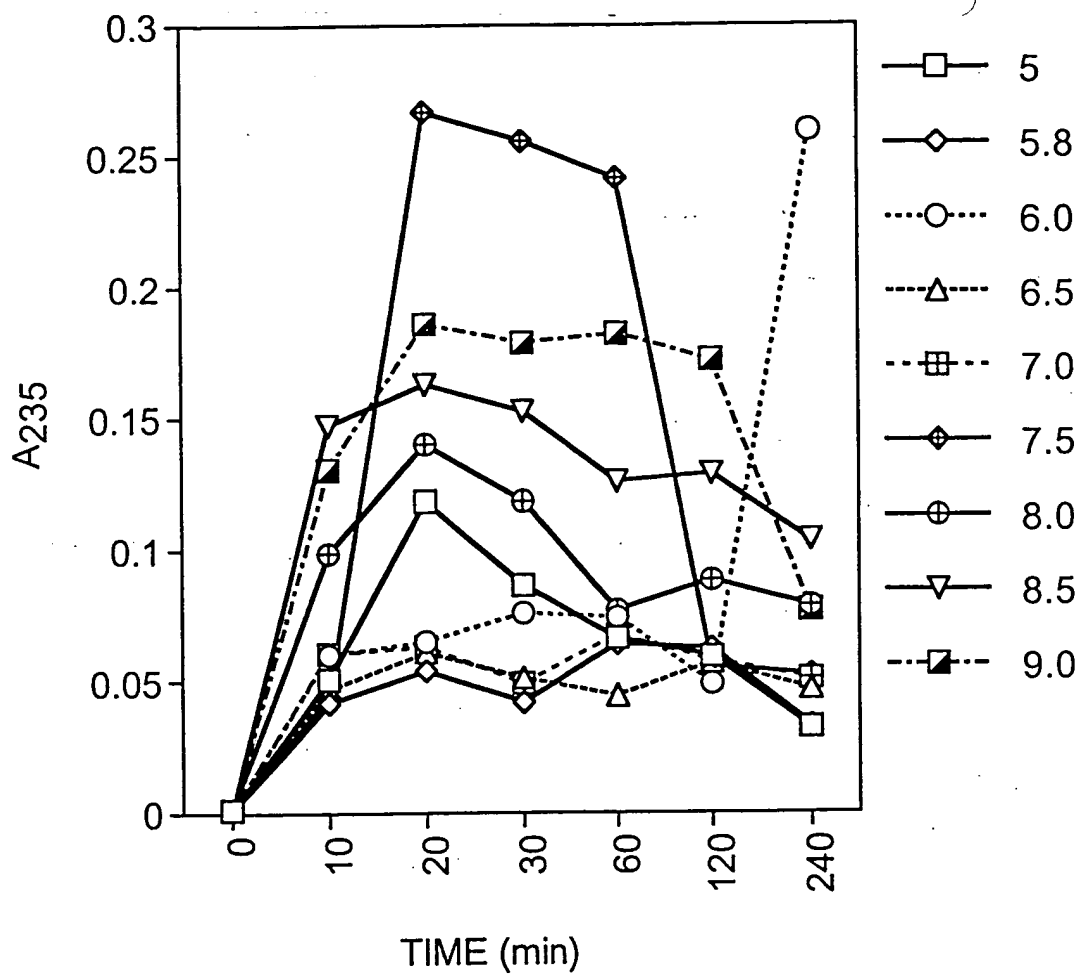


FIG. 27

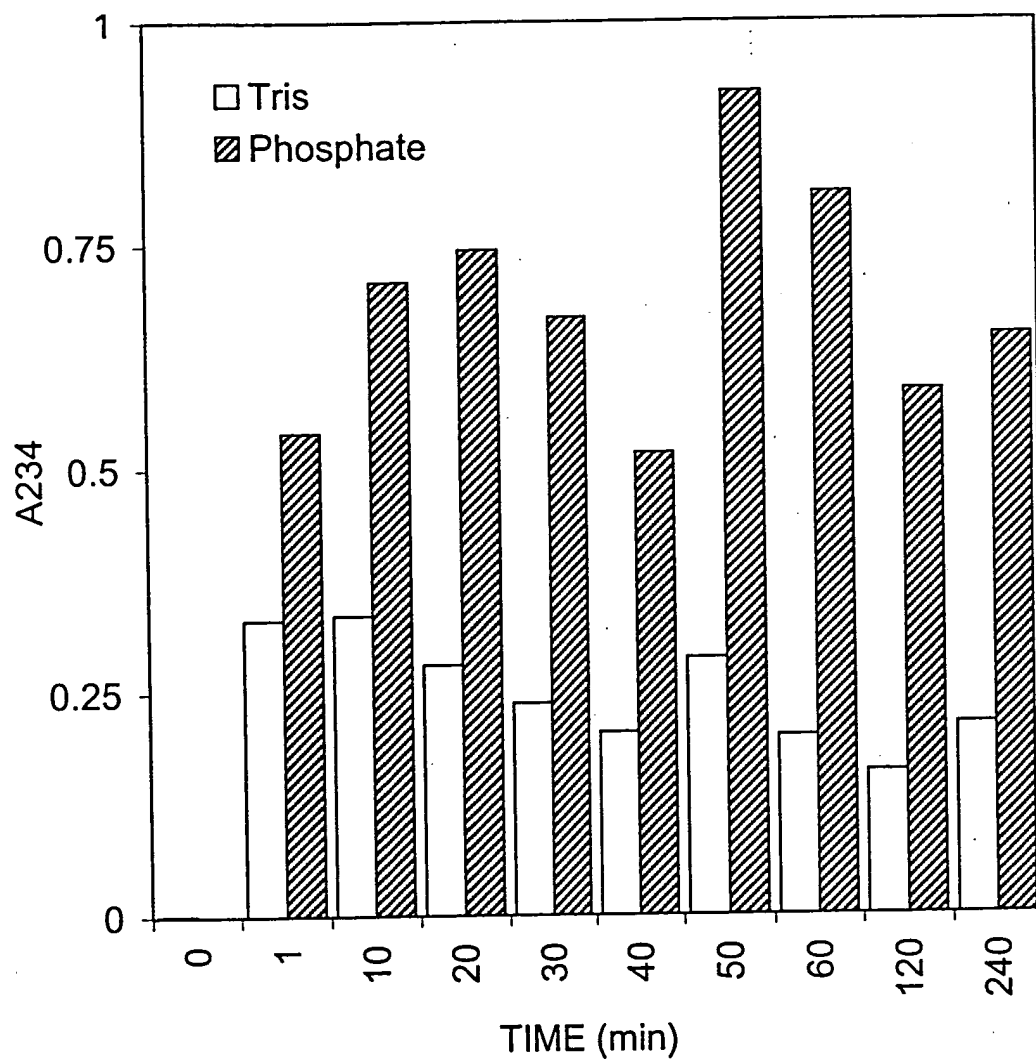


FIG. 28

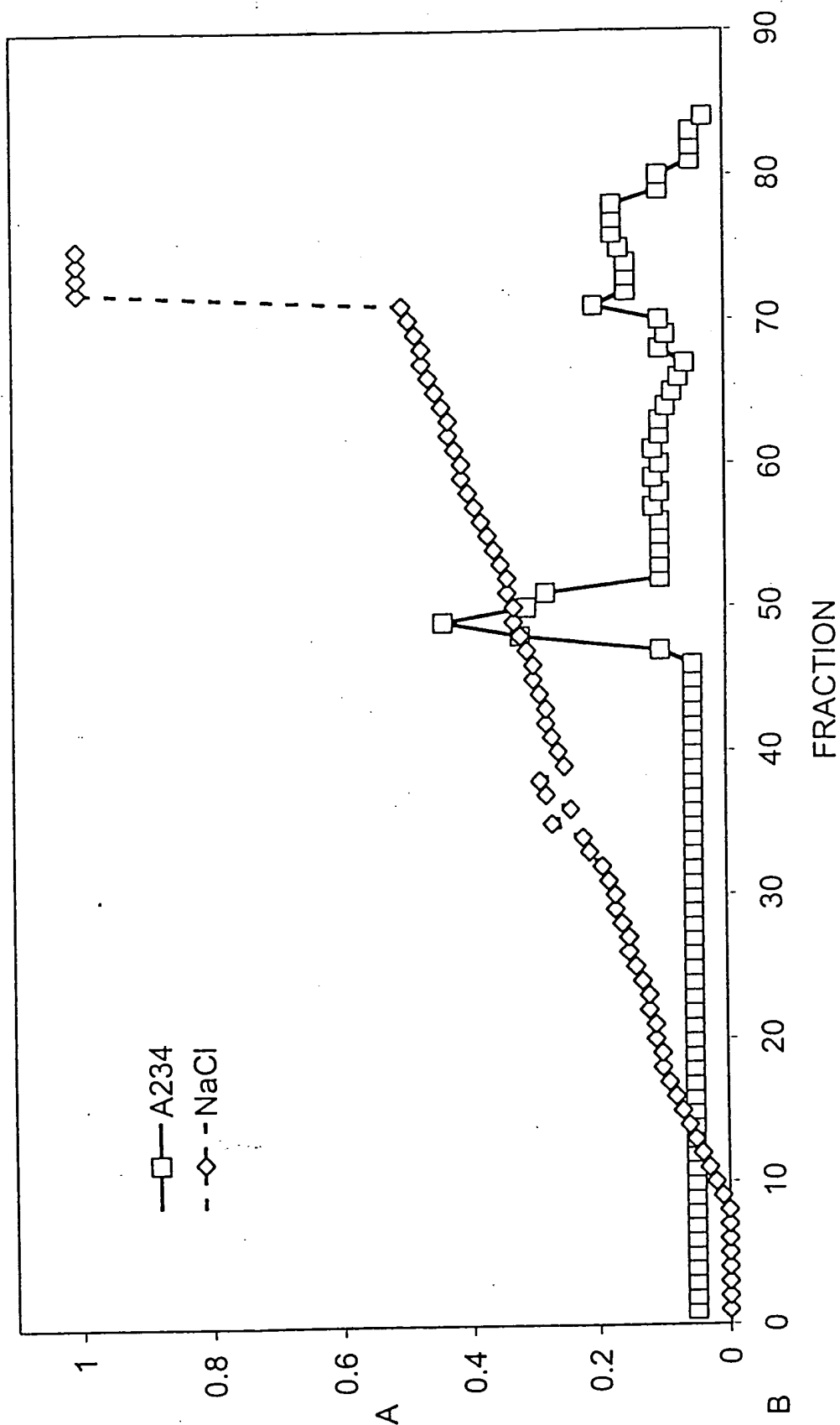


FIG. 29

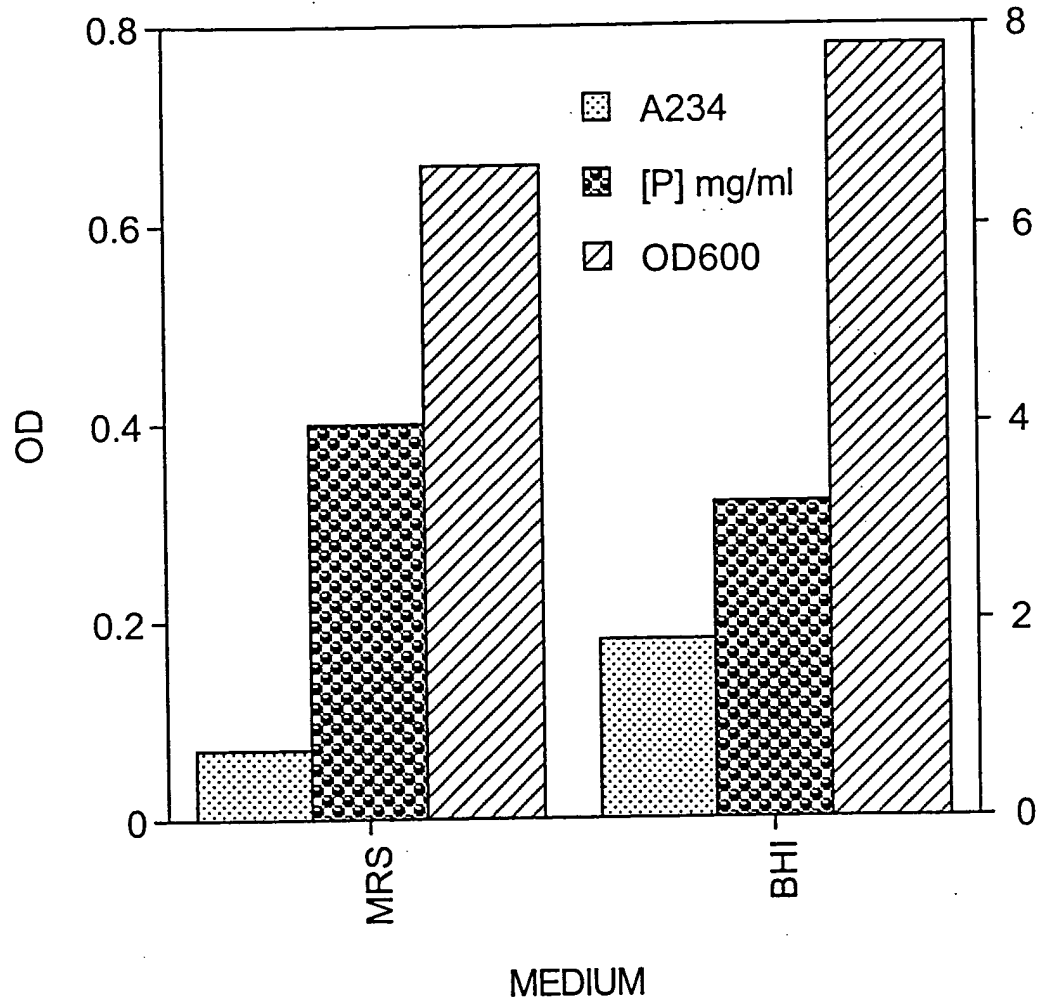


FIG. 30

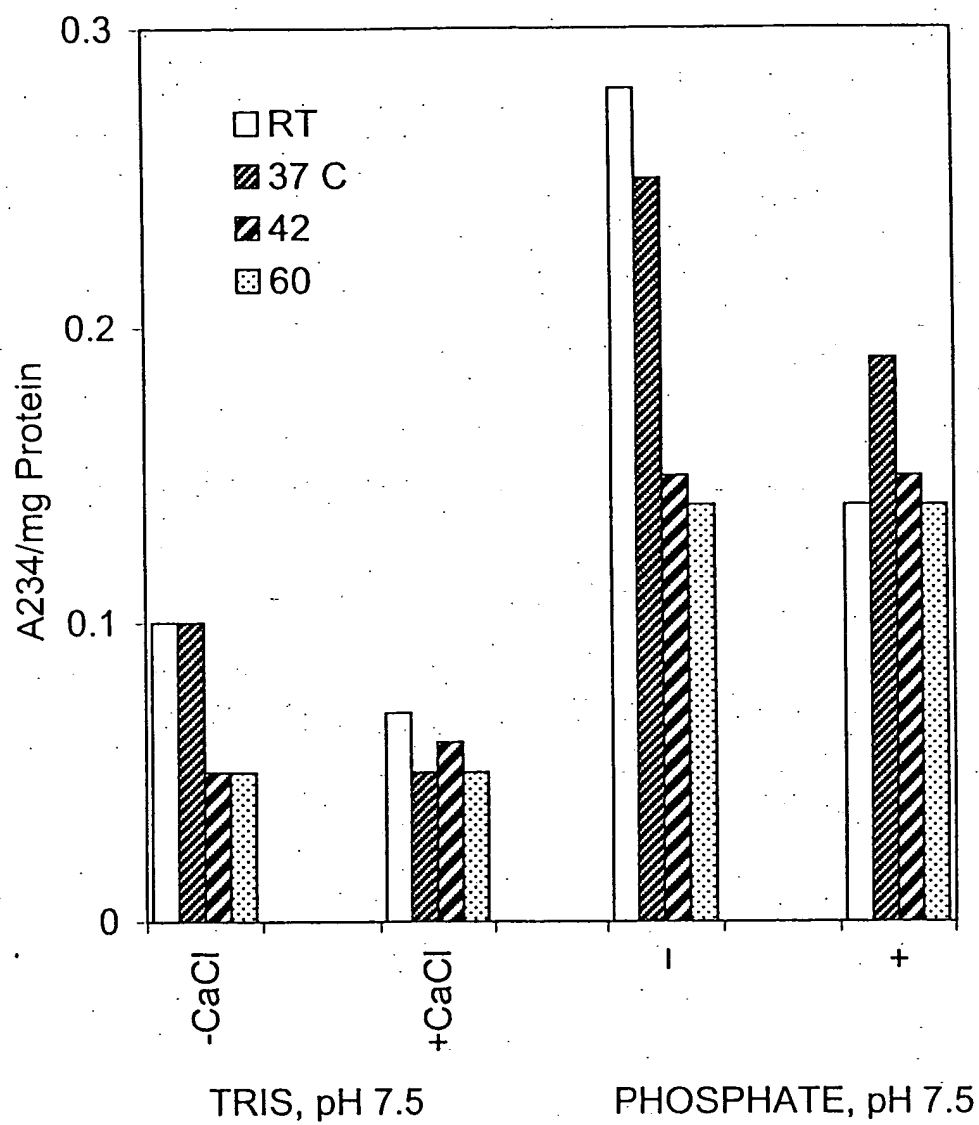


FIG. 31



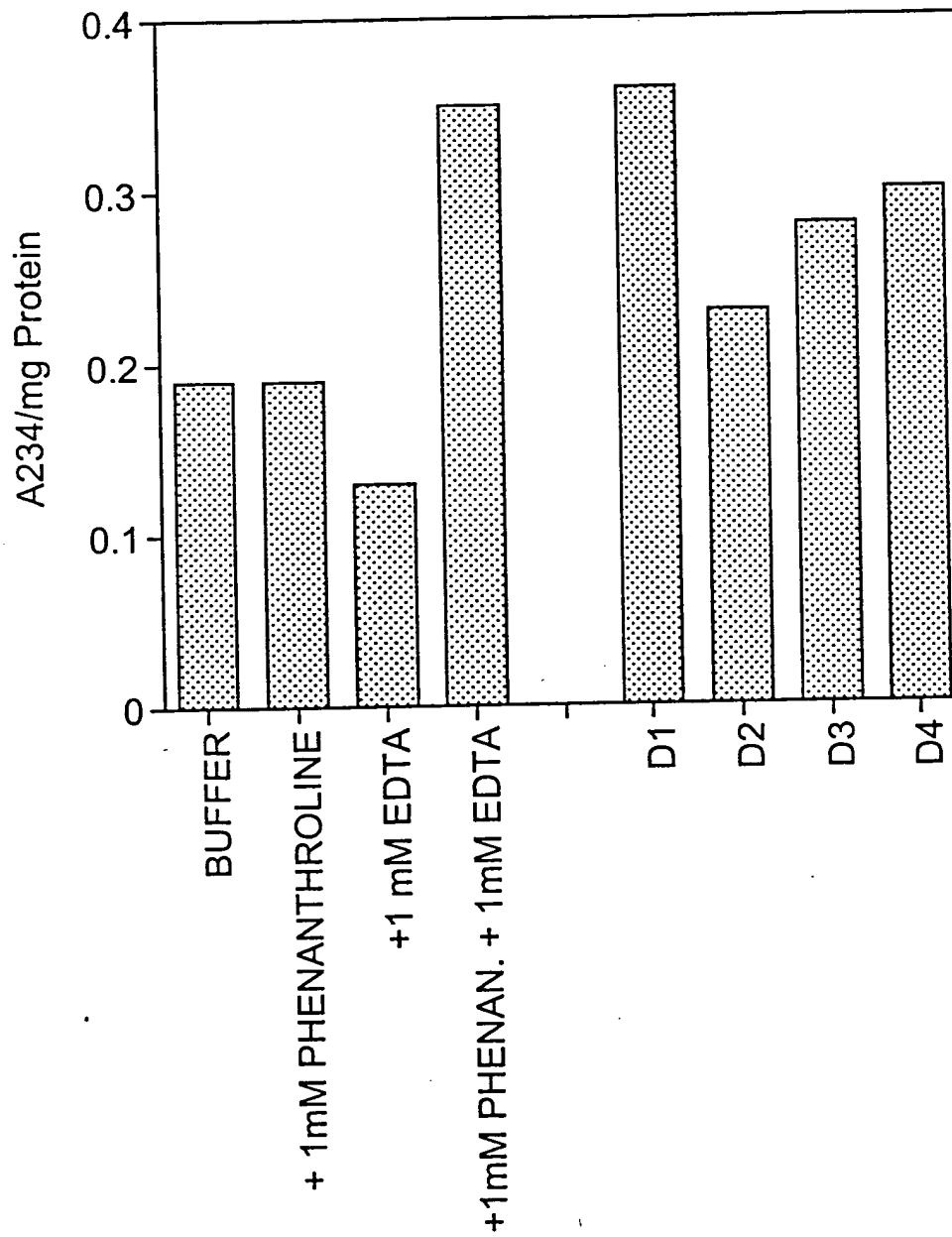


FIG. 32

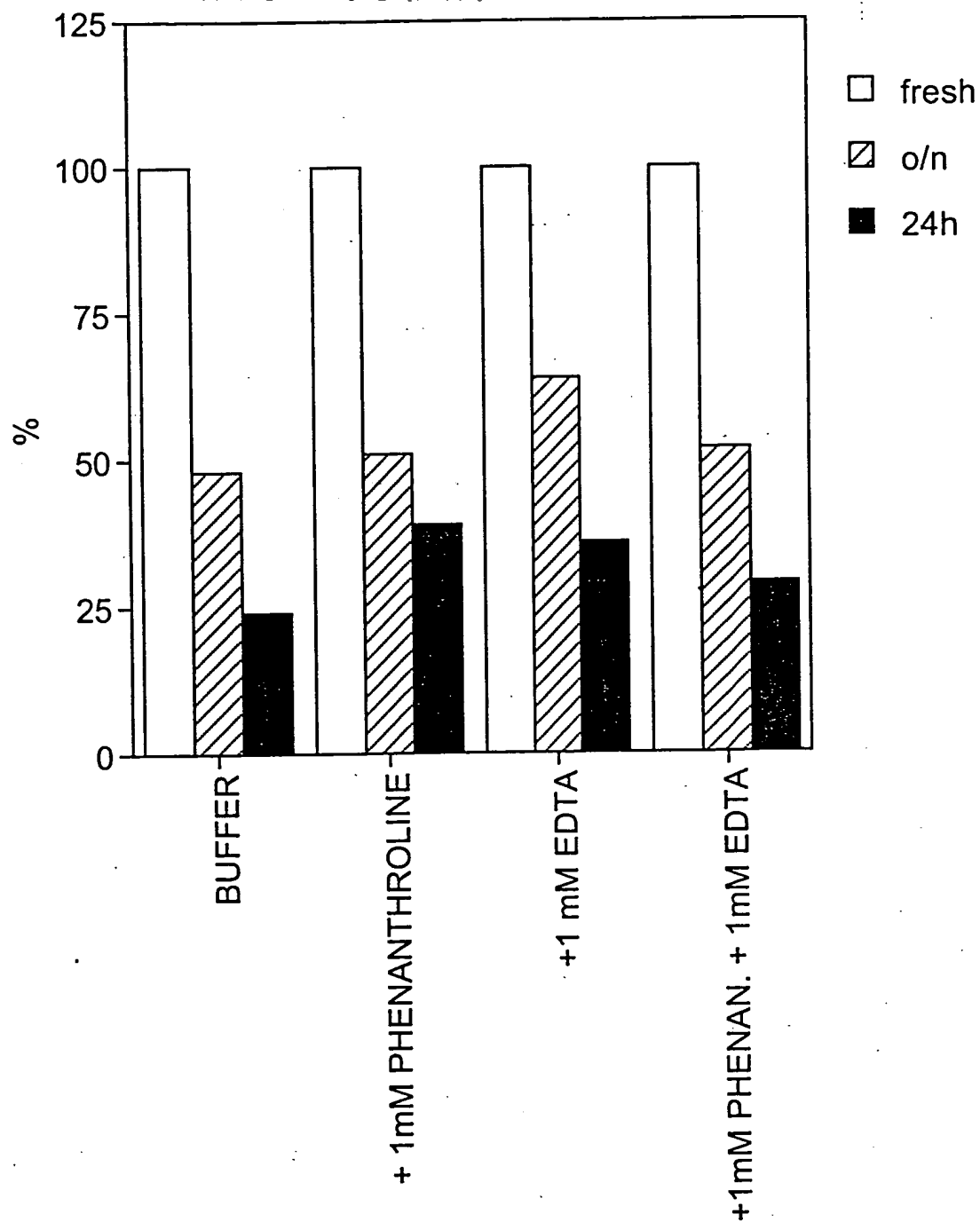


FIG. 33

### pH Effect on Extraction Efficiency of Isomerase

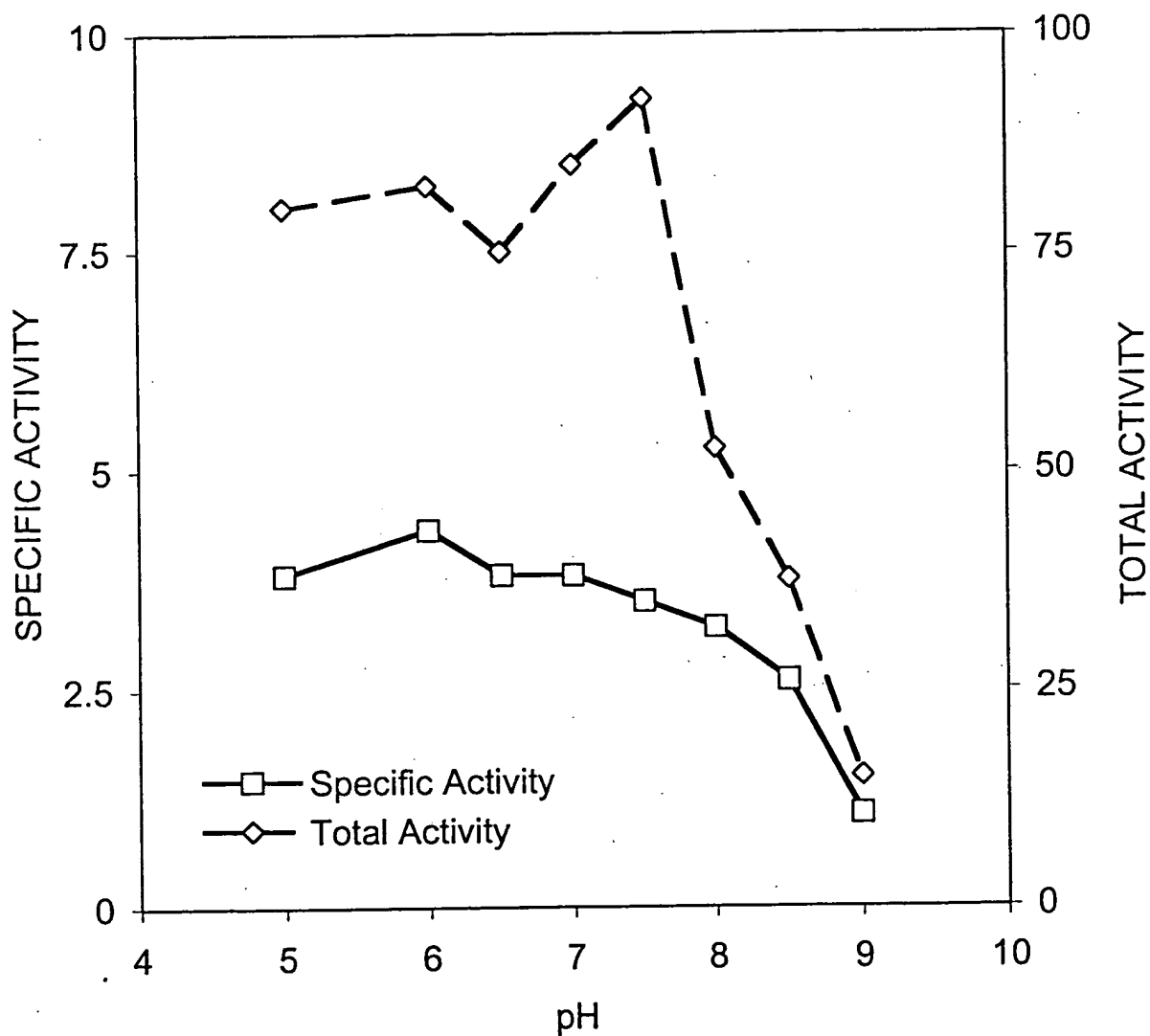


FIG. 34

### Half Lives of Isomerase vs pH

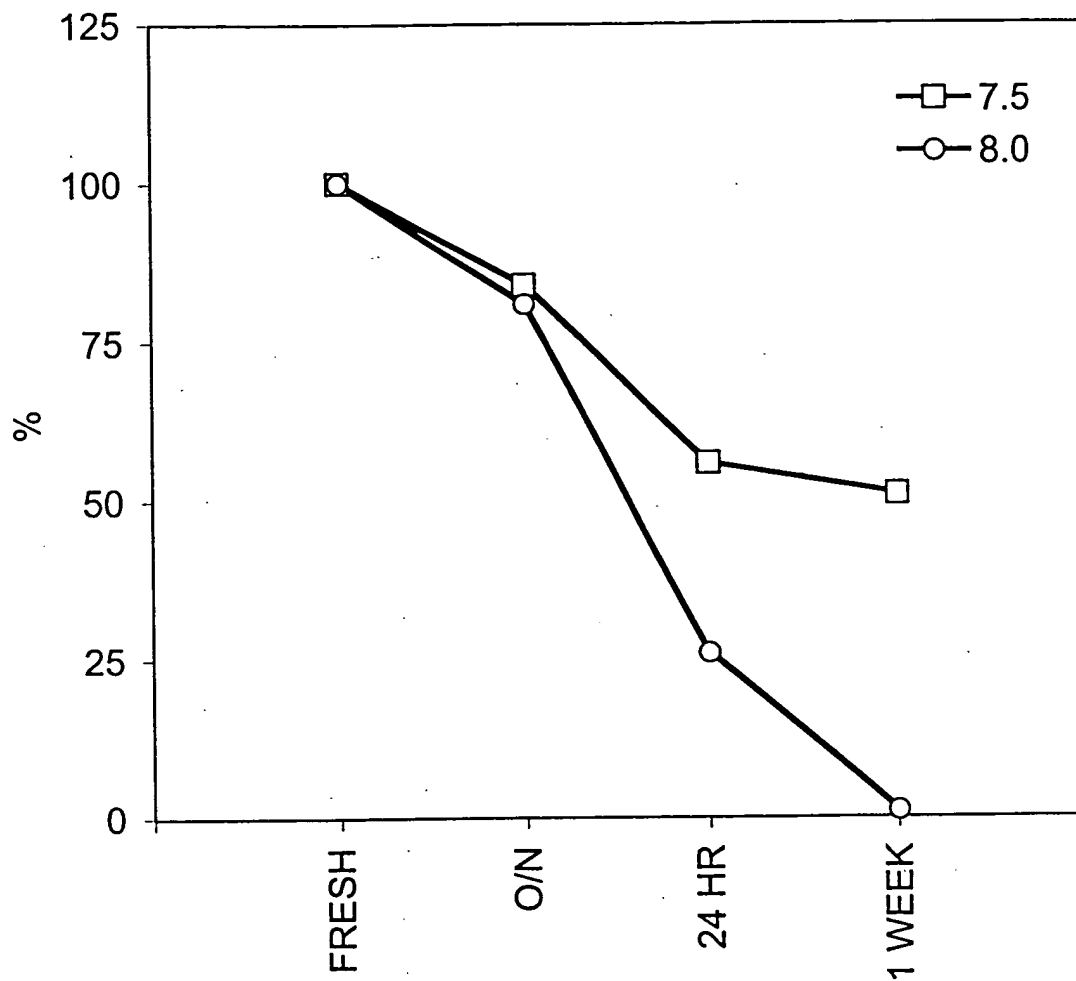


FIG. 35

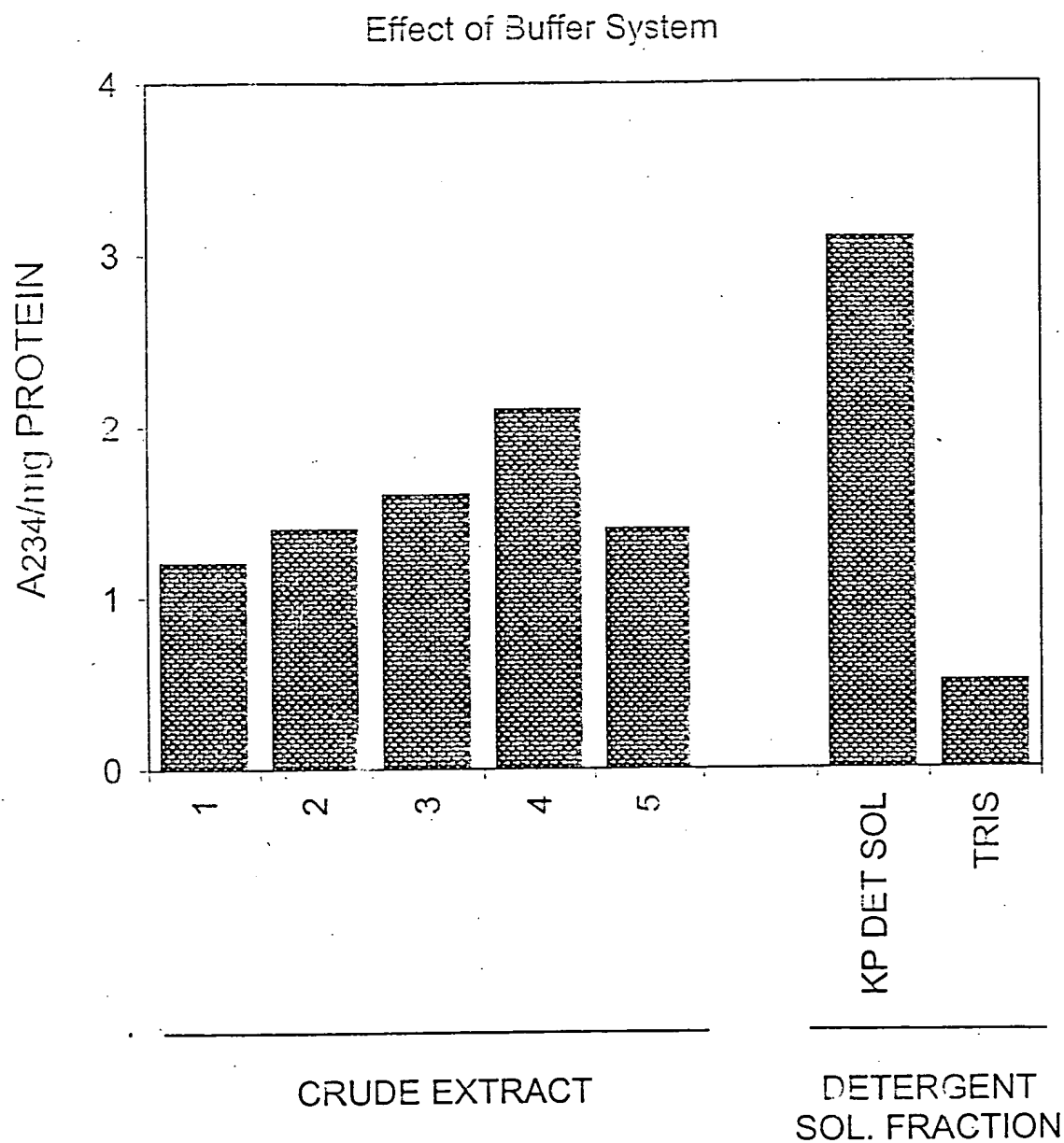


FIG. 36

### Effect of Glycerol and Salt Concentration on Isomerase Stability

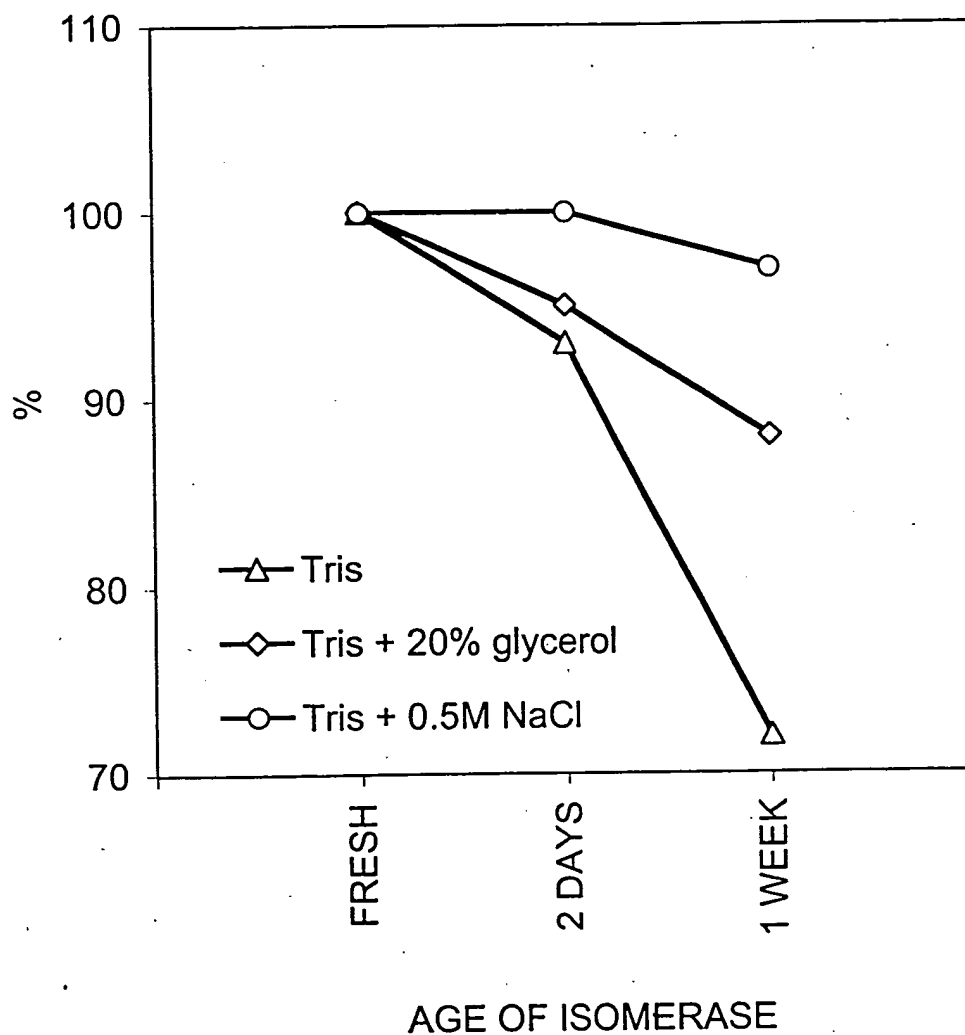


FIG. 37

### Stability of Detergent Solubilized Isomerase

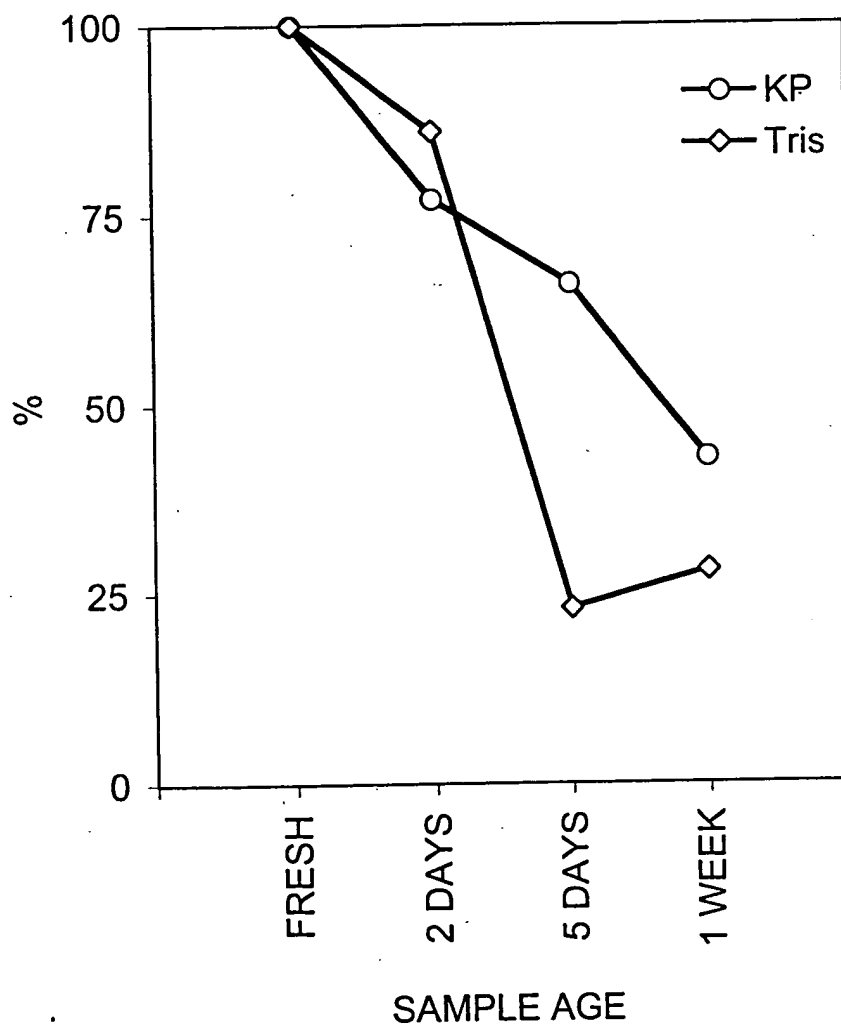


FIG. 38

### Chromatography on Mono Q Column

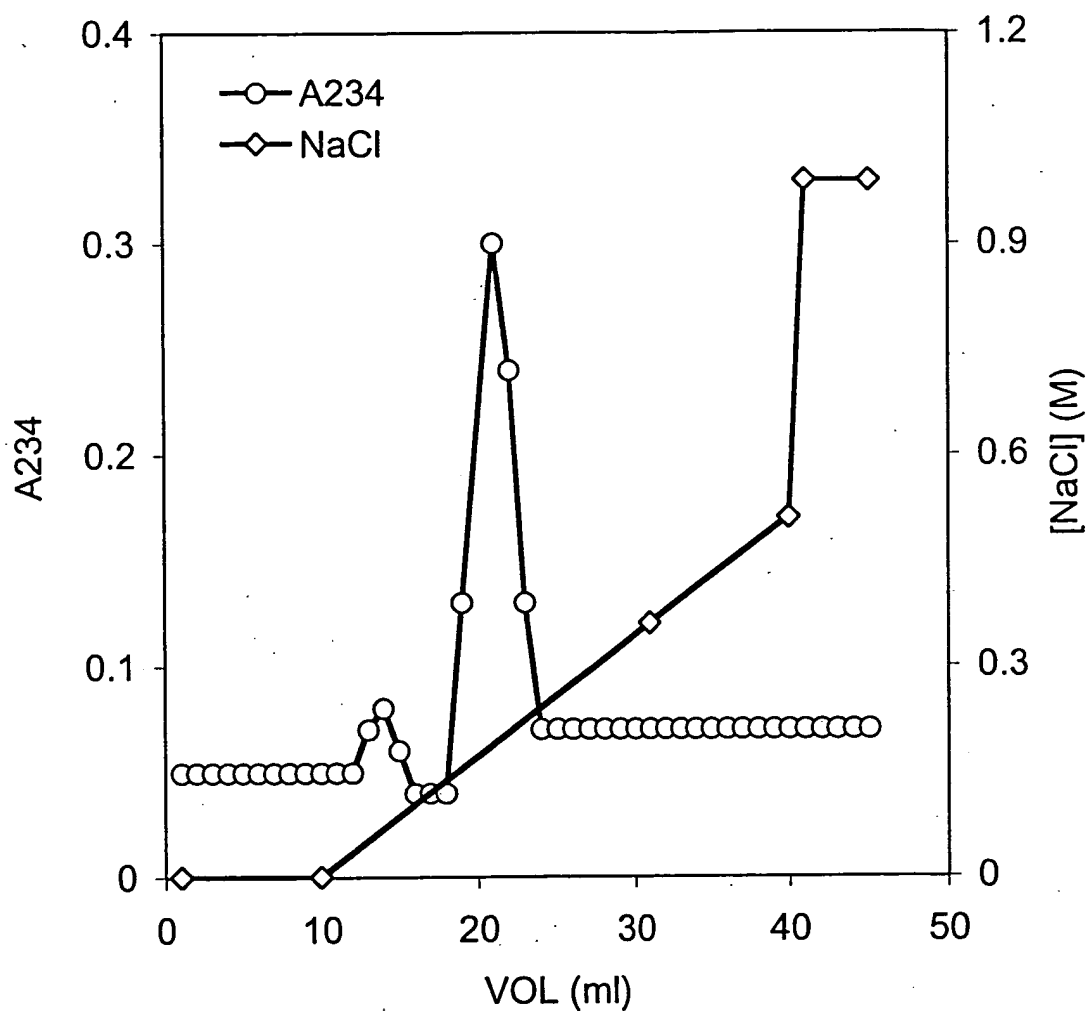


FIG. 39



### Separation of Partially Purified Isomerase by Chromatofocusing

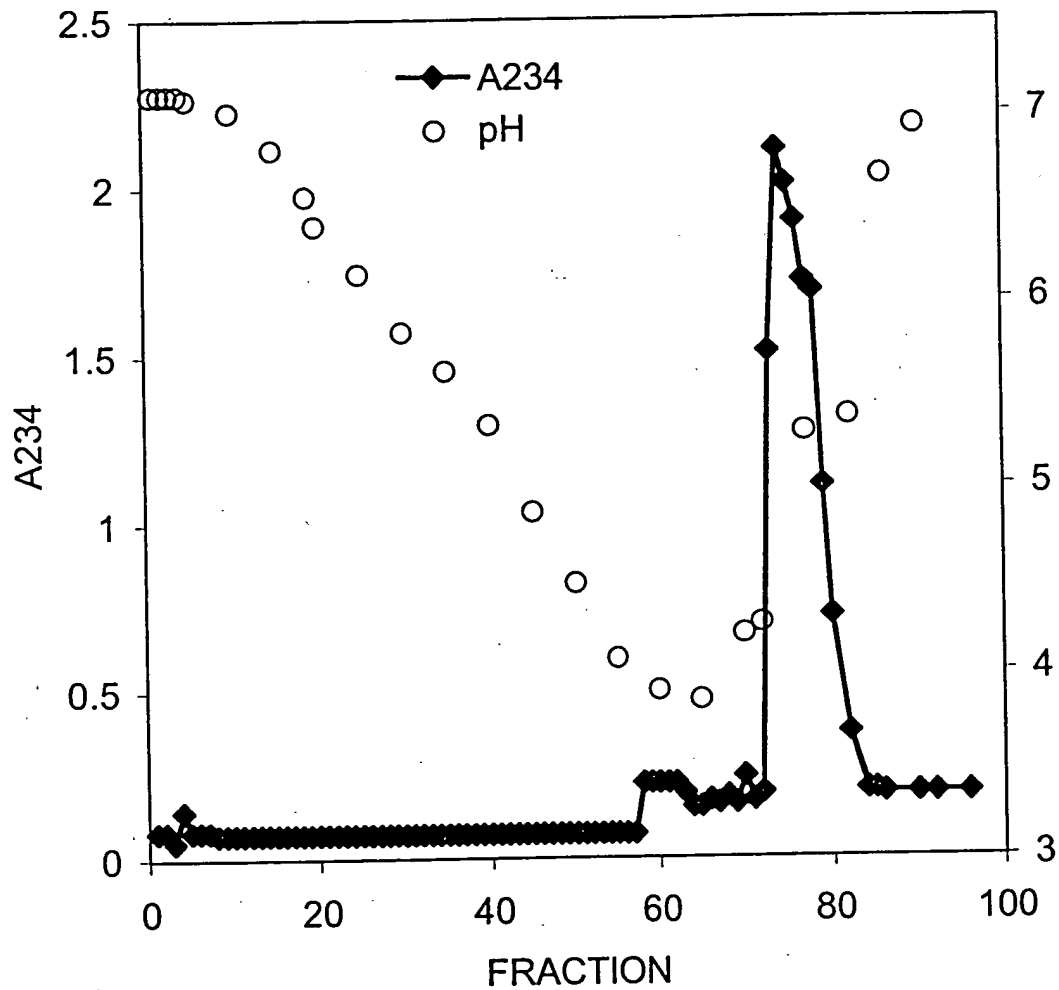


FIG. 40

### Chromatography of Detergent Solubilized Isomerase on DEAE-5PW Column

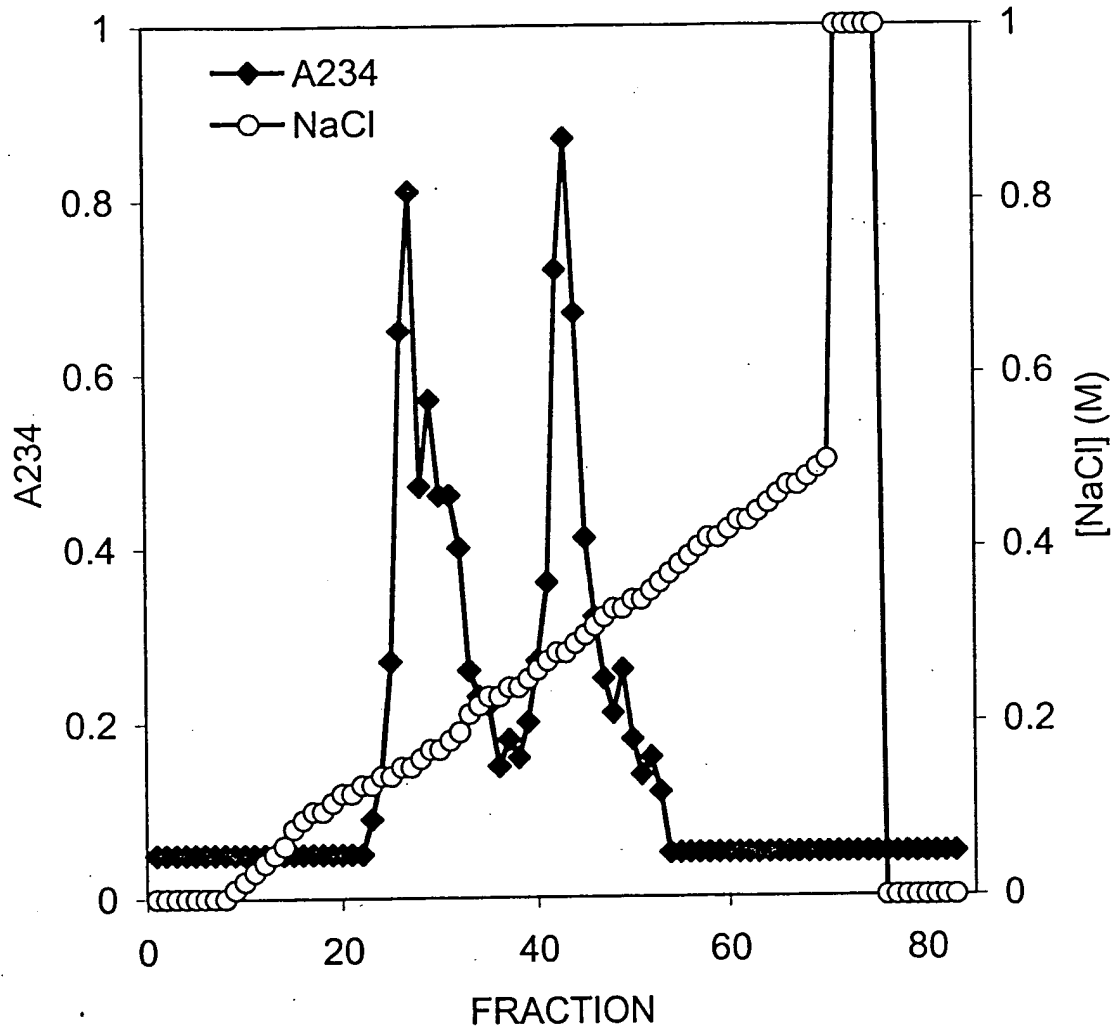
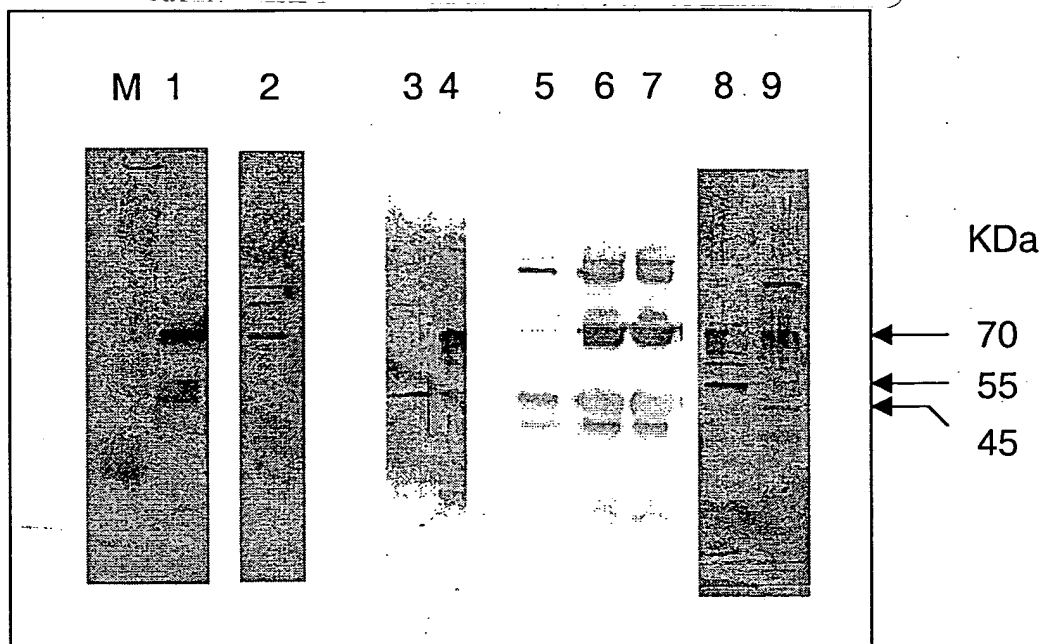


FIG. 41

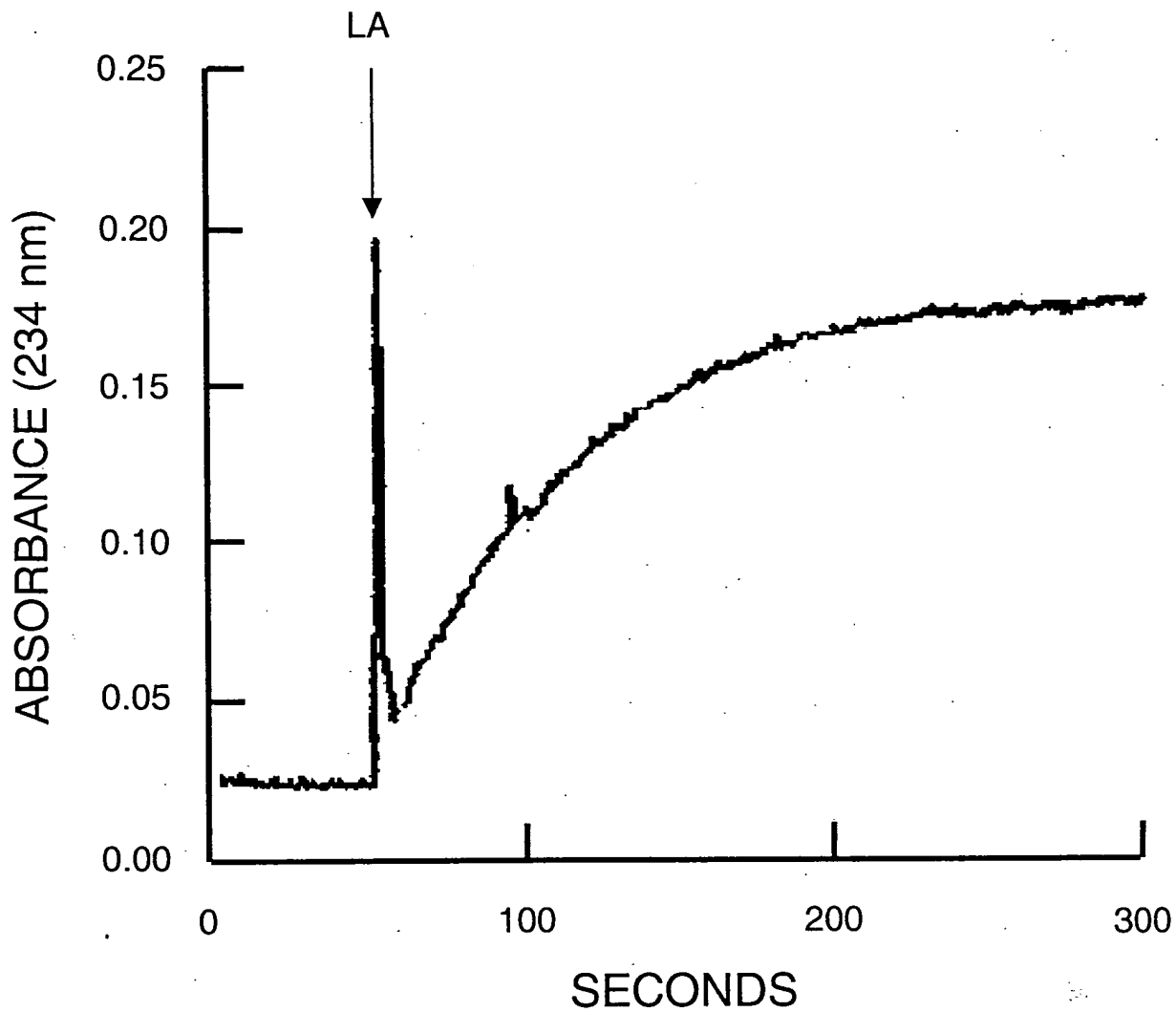


Western Blot analysis of linoleate isomerase using rabbit antibodies specific for the cloned *L. reuteri* PYR8 isomerase. Total protein of cell lysates prepared from different strains was used in the analysis.

M. Protein size marker

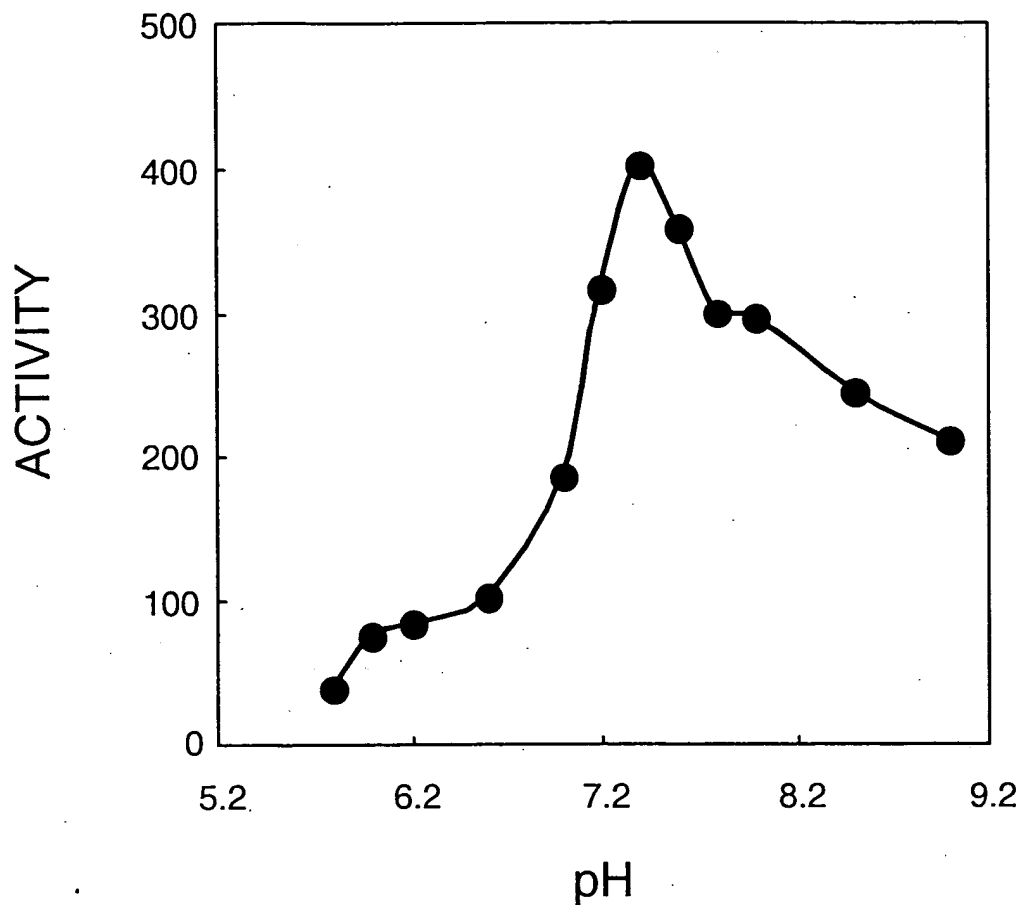
1. *E. coli* expressing the isomerase-histag fusion protein
2. *L. reuteri* PYR8
3. *B. subtilis* wild type
4. *B. subtilis* transformed with the vector pBH1 containing the isomerase gene under HpaII promoter control
5. *L. reuteri* 23272 wild type
6. *L. reuteri* 23272 transformed with the vector pTRKH2 containing the isomerase gene under the control of both its native promoter and the *lac* promoter
7. *L. reuteri* 23272 transformed with the vector pTRKH2 containing the isomerase gene under the control of its native promoter
8. *P. acnes* 6919
9. *C. sporogenes* 23272

FIG. 42



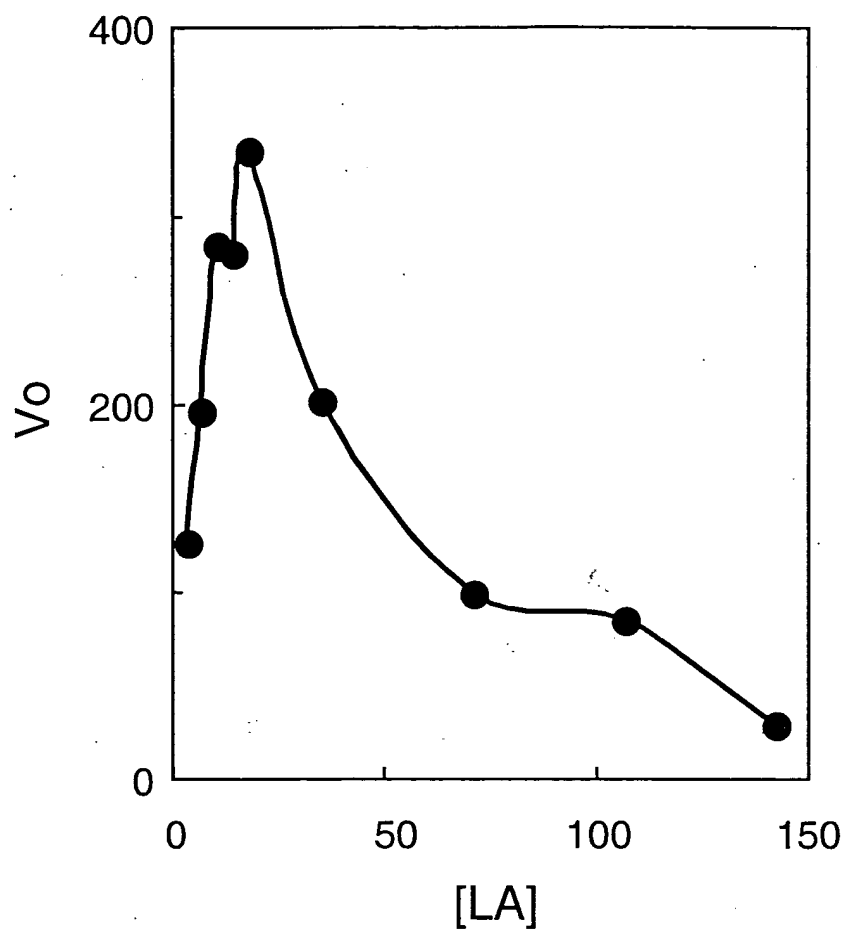
Time course of isomerization of linoleic acid. The isomerization reaction was initiated by adding 20  $\mu$ M linoleic acid at 50 seconds.

FIG. 43



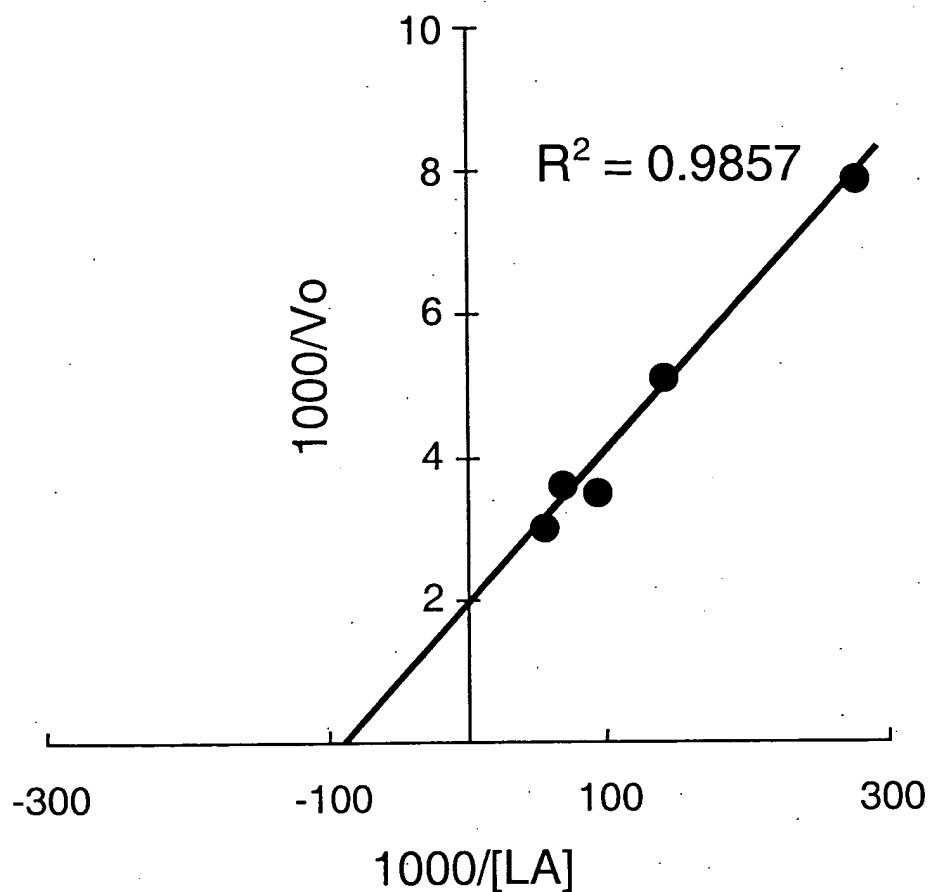
Effect of pH on isomerization of linoleic acid to CLA by  
*C. sporogenes* linoleate isomerase. Activity as nmol CLA/min/mg protein.

FIG. 44



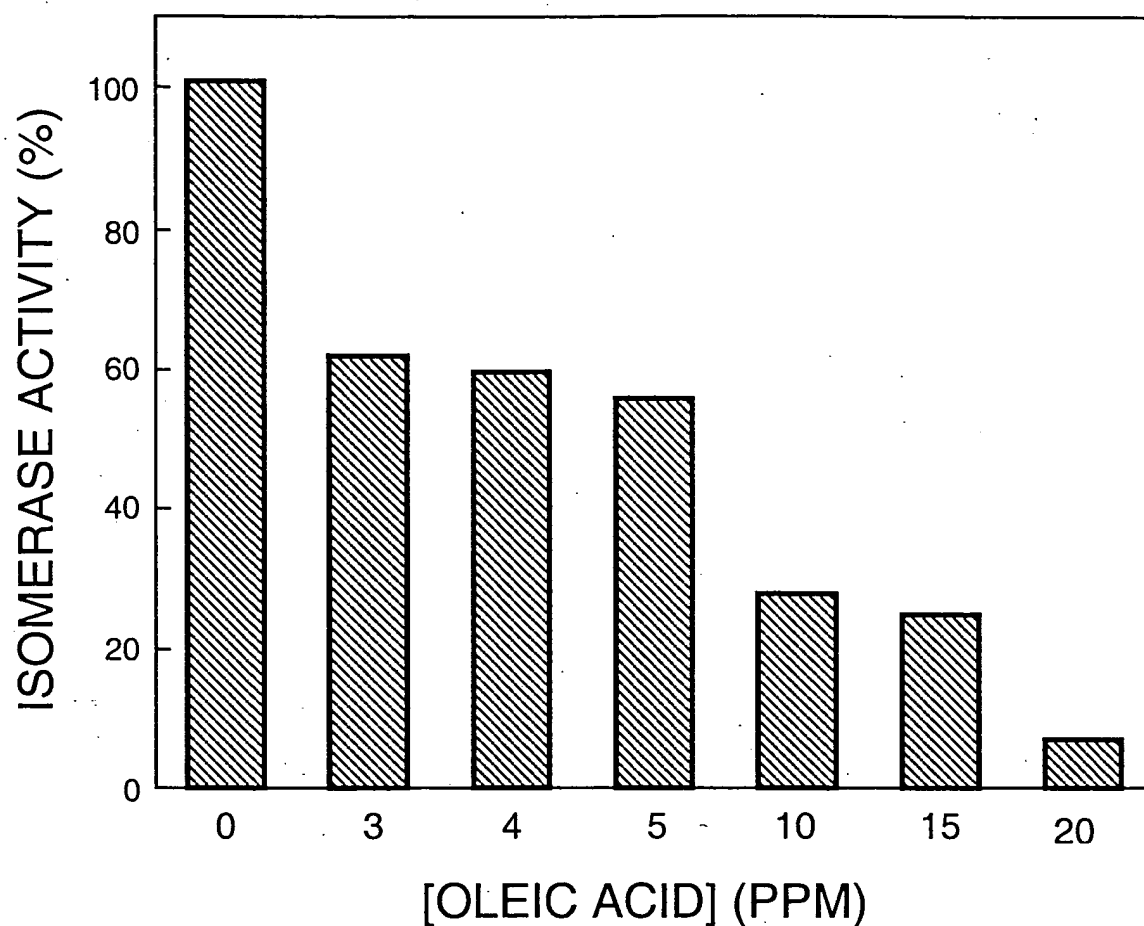
Effect of linoleic acid concentration on the rate of isomerization.  
Vo as nmols CLA/min/mg protein. [LA] as μM.

FIG. 45



Lineweaver-Burk plot of reaction kinetics of *C. sporogenes* linoleate isomerase.  $V_o$  as nmols CLA/min/mg protein.  $[LA]$  as  $\mu M$ .

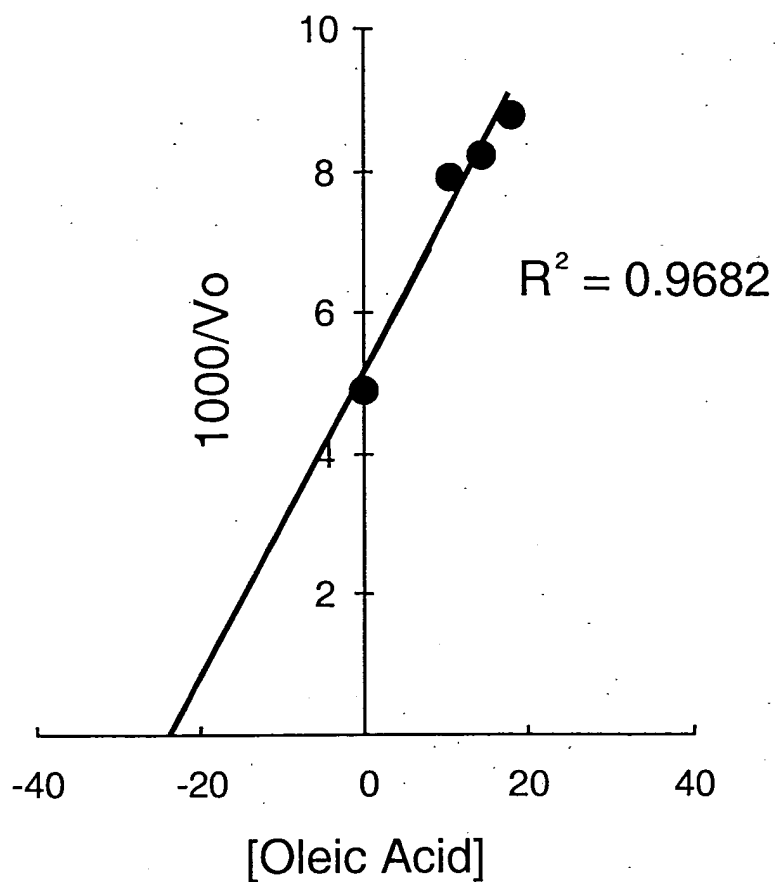
FIG. 46



Effect of oleic acid on isomerase activity with linoleic acid as substrate. The concentration of linoleic acid was fixed at 36  $\mu$ M. Oleic acid was added at the indicated concentrations.

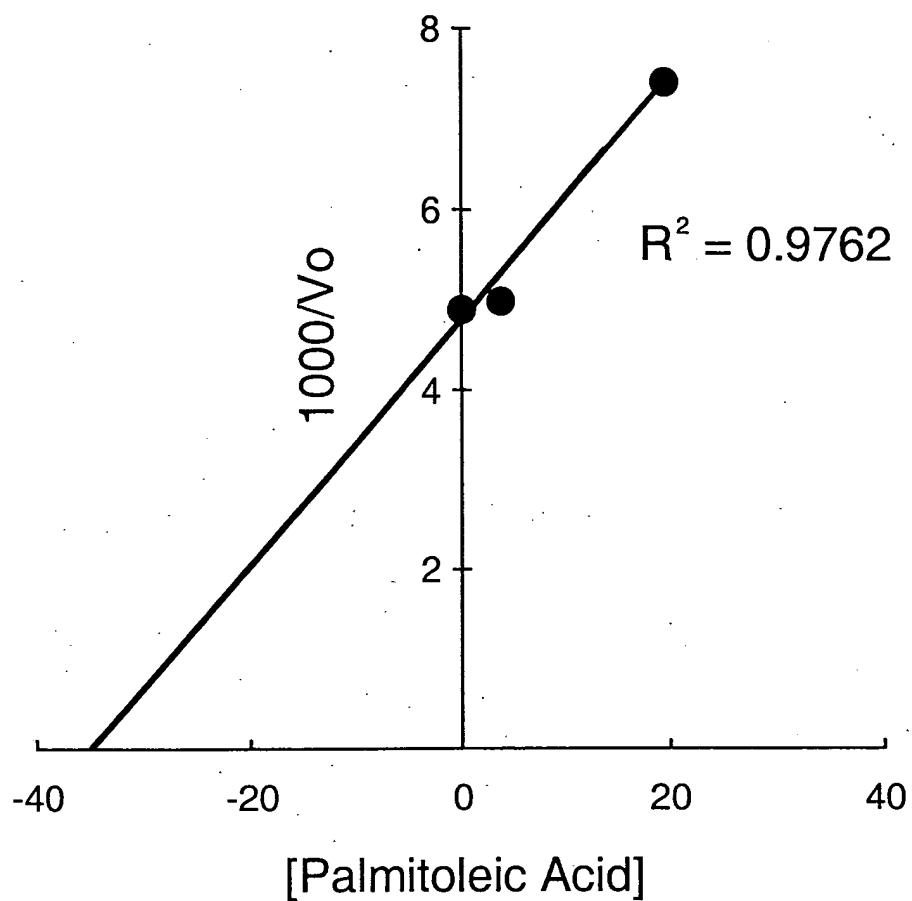
FIG. 47





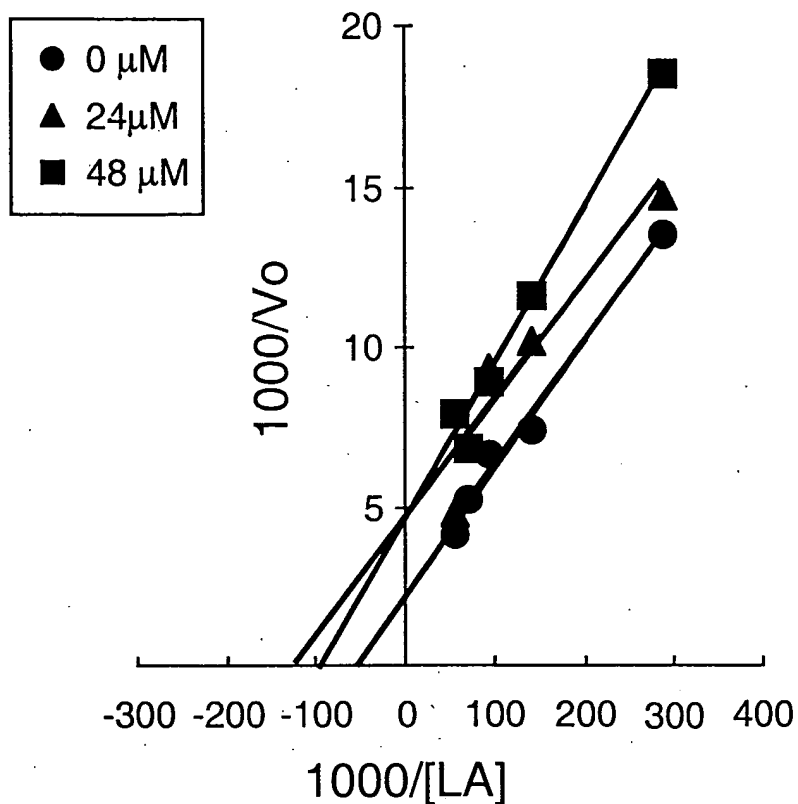
Secondary plot of oleic acid inhibition.  $V_o$  as nmols.  
CLA/min/mg protein.  $[Oleic\ Acid]$  as  $\mu M$

FIG. 48



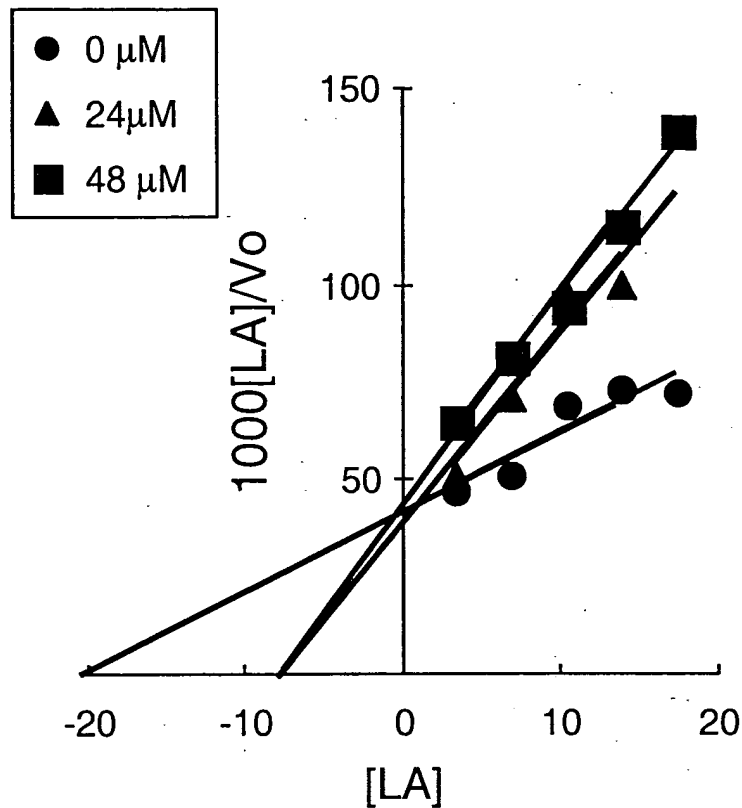
Secondary plot of palmitoleic acid inhibition.  $V_o$  as nmols  
CLA/min/mg protein.  $[Palmitoleic\ Acid]$  as  $\mu M$

FIG. 49



Lineweaver-Burk plot of linoleic acid isomerization kinetics in the presence or absence of oleic acid.  
 $V_o$  as nmols CLA/min/mg protein. [LA] as  $\mu M$ .

FIG. 50



Hanes-Woolf plot of oleic acid inhibition of linoleic acid isomerization kinetics.  $V_o$  as nmols CLA/min/mg protein.  $[LA]$  as  $\mu\text{M}$ .

FIG. 51

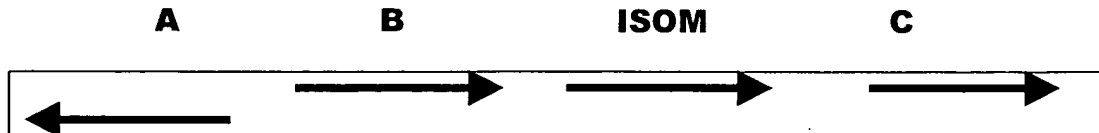


FIG. 52

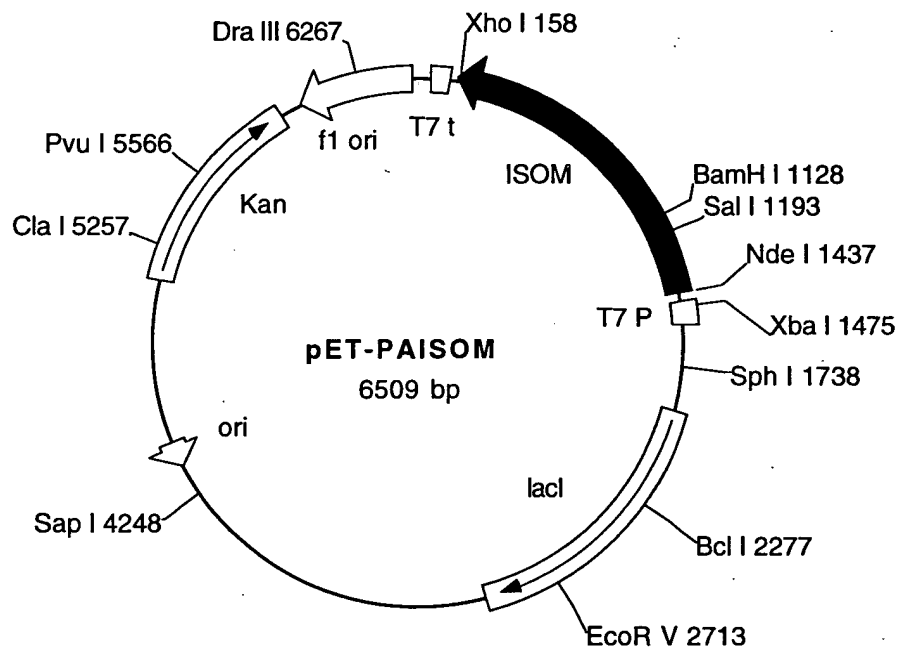


FIG. 53

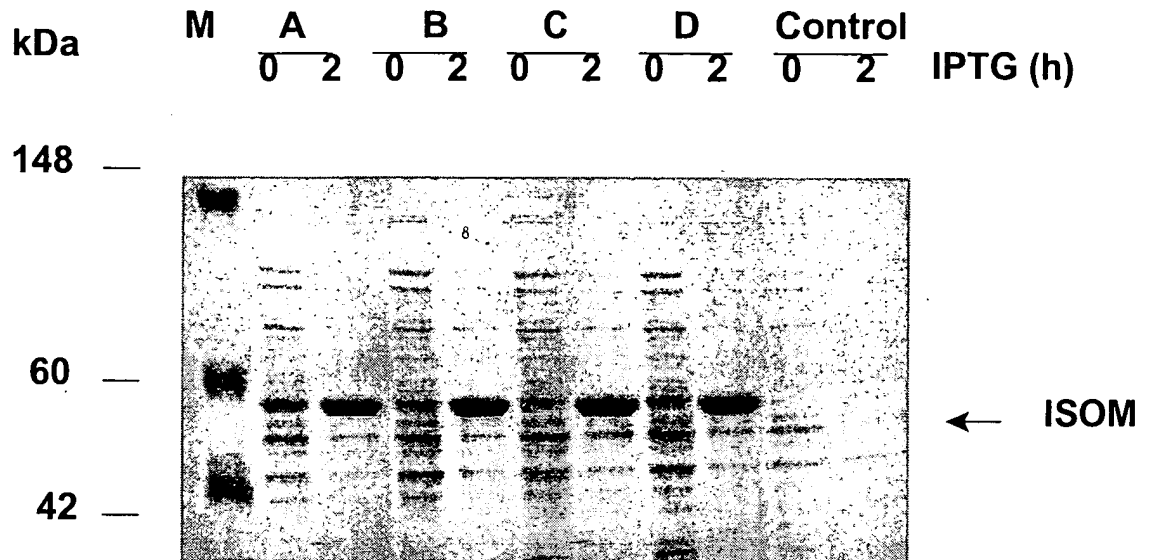


FIG. 54

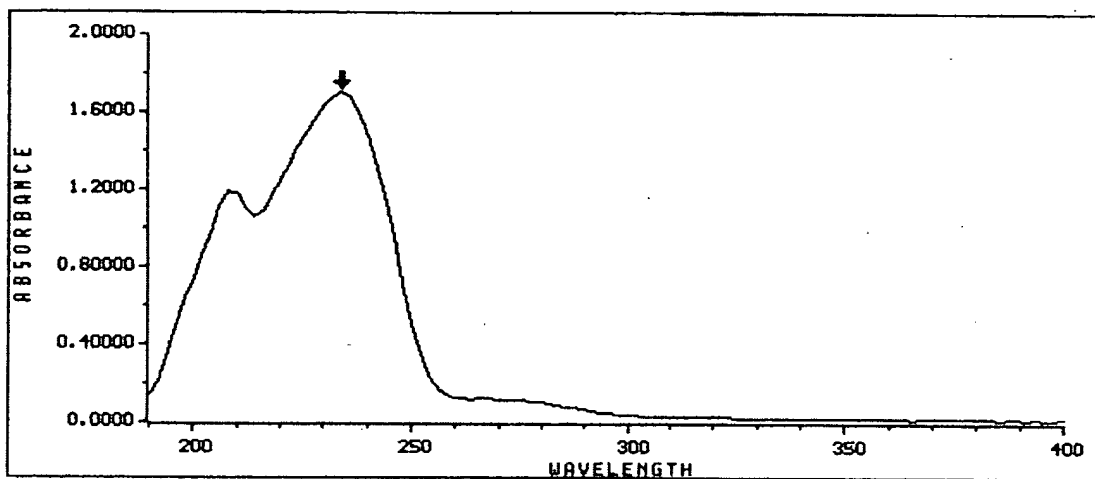


FIG. 55



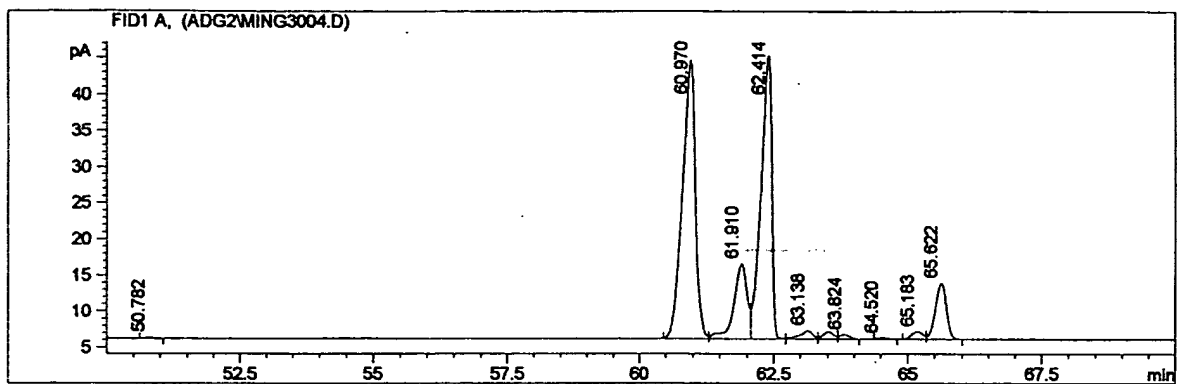


FIG. 56A

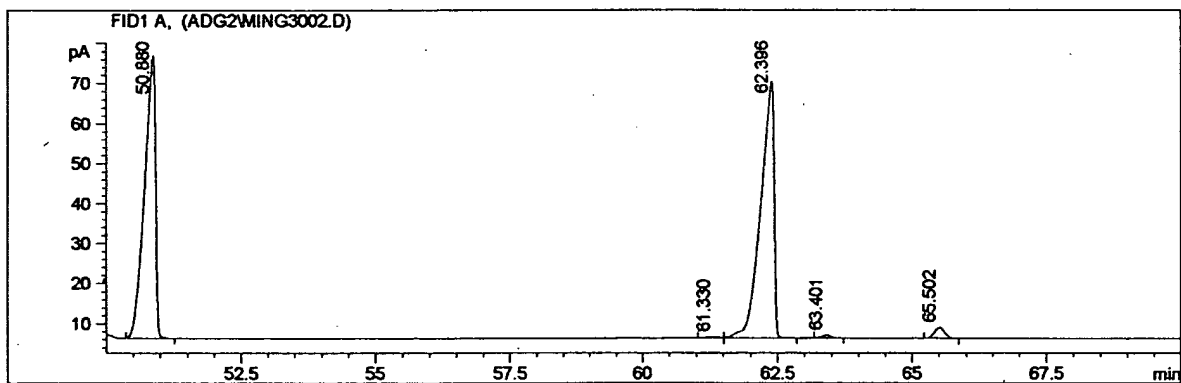


FIG. 56B

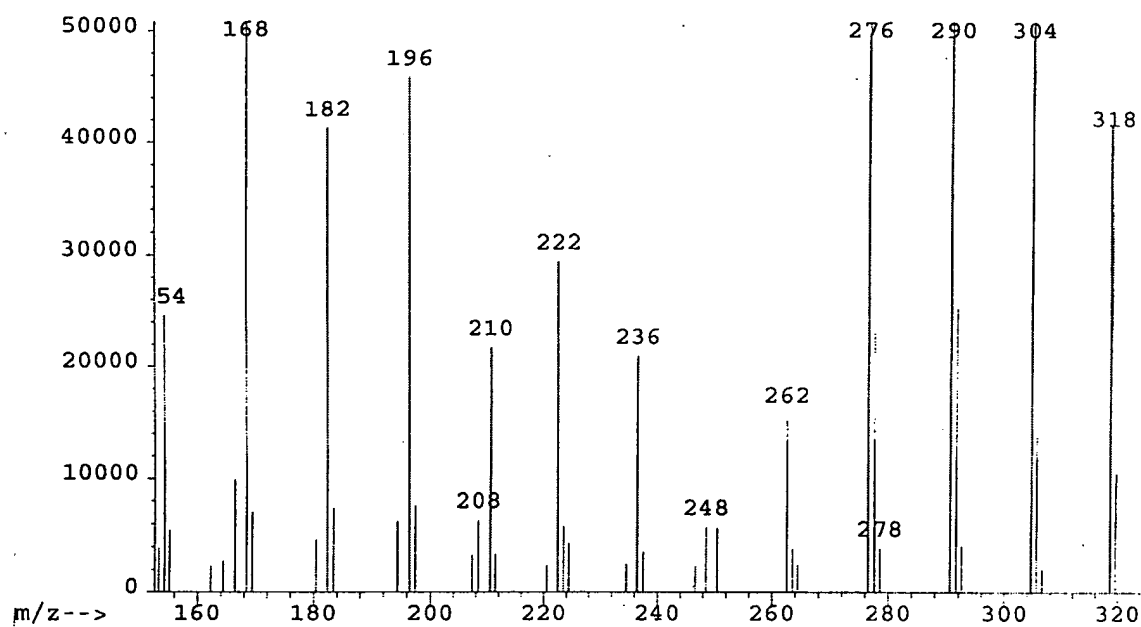
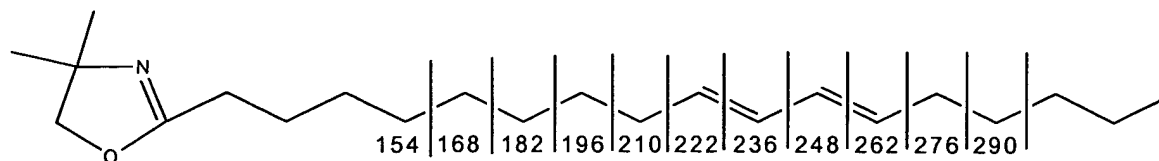


FIG. 57

Title of Invention: "LINOLEATE ISOMERASE"  
Inventor's Name: ROSSON et al.  
Serial No. Not Yet Assigned  
Docket No. 3161-20-C1-1  
Agent: Angela Dallas Sebor  
Telephone: 303/863-9700

**NAD-binding domain**

**SEQUENCE ORIGIN:**

FIG. 58